The Journal of Laboratory and Clinical Medicine

VOL. 22 APRIL, 1937 NO. 7

CLINICAL AND EXPERIMENTAL

CONCERNING THE IDENTITY OF THE ANTIBODY IN EXPERIMENTAL ANAPHYLAXIS AND THAT OCCURRING IN MAN NATURALLY OR SPONTANEOUSLY SENSITIZED*

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IT IS not our purpose in this communication to cite the literature, or discuss the relative merits of the views of those who advocate essential differences or similarities for the state of anaphylaxis, experimentally induced in animals, and those clinical conditions in man, spontaneously arising and termed allergic. We wish, however, to present the results of three sets of Prausnitz-Kustner reactions which have direct bearing on these issues.

In a previous publication† we have shown that all guinea pigs injected with ragweed pollen extract to which potassium alum had been added developed an unusually high degree of sensitivity. The state of anaphylaxis so induced was such that all of the animals on reinjection with an appropriate shocking dose promptly developed severe anaphylactic shock and in most instances death ensued.

We have found this method of sensitization so reliable that it relieves one of the necessity of demonstrating anaphylactic shock in each individual animal.

Guinea Pig 503 was one of a group sensitized by the subcutaneous injection of 2.5 c.c. of alum precipitated ragweed extract 91134 on March 19, 1936. On June 16, 1936, 5 c.c. of blood were withdrawn from the heart. On June 27,

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Received for publication, August 22, 1936.

†Caulfeild, Brown, and Waters: Alum as an Adjuvant in Sensitizing Guinea Pigs to Ragweed Pollen, J. Allergy 7: 451, 1936.

1936, the intravenous injection of the guinea pig with 0.75 c.c. of ragweed extract without alum caused typical and immediate anaphylactic death which was confirmed by autopsy.

The serum (from Guinea Pig 503), undiluted and diluted 1/10, was injected intradermally into the back of a suitable human recipient (or test subject). At the same time, control injections were made of undiluted and diluted 1/10 normal guinea pig serum, undiluted normal human serum, and serial dilutions, up to 1/100, of serum from a case (McL.) of ragweed hay

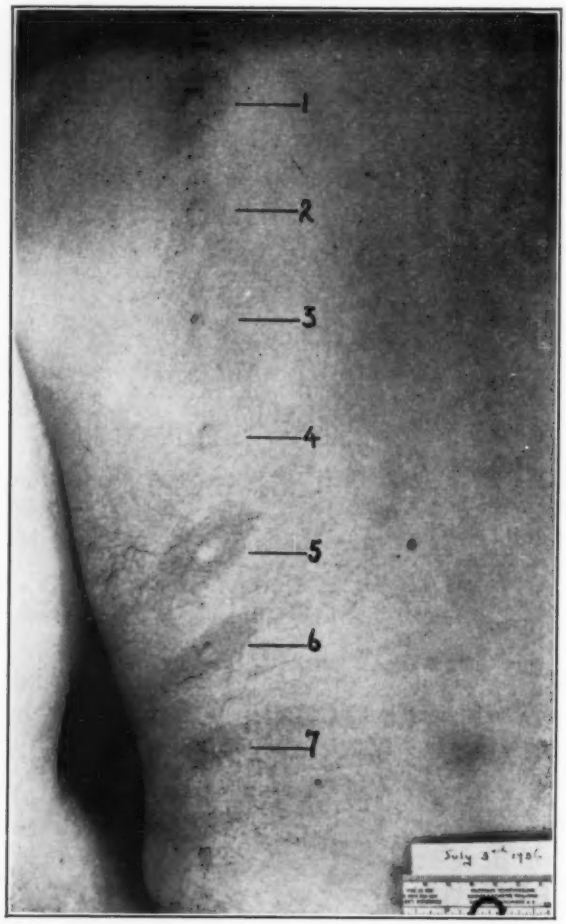

Fig. 1A.

fever. The following day these sites were reinjected with 0.05 c.c. of 1/100[1] dilution of watery extract (W.E.) of ragweed. As is shown in Table I, both the serum from Guinea Pig 503, and the serum from the case of ragweed hay fever (McL.) induced local passive sensitization. All the controls were negative as may be seen in the tabulated results and the Figs. 1*A* and *B*.*

*At this time of year it is difficult to obtain recipients who have not sun-tanned backs. The individuals used in these experiments were well browned. In consequence, the photographs do not show the contrast between normal skin and the areola, with its central wheal, of a positive reaction as clearly as they usually do. Photographs of a group of skin reactions more frequently than not misrepresent more or less one or more sites. This is more likely to occur with varied areas of sunburning. In Tables I and II plus signs are used taken from the results shown by serial dilutions of human serum. In Tables III and IV the human serum was not diluted and consequently we have given millimeter measurements of the areolas.

The only apparent difference between the results obtained with the guinea pig Serum 503 and the human serum (McL.) used in this experiment, and as well with that used in the second, is that there is a greater concentration of antibody in the human serum. We have, however, found among the serums taken from many cases of ragweed hay fever and titrated in a similar fashion with different recipients, examples of all grades of concentration including

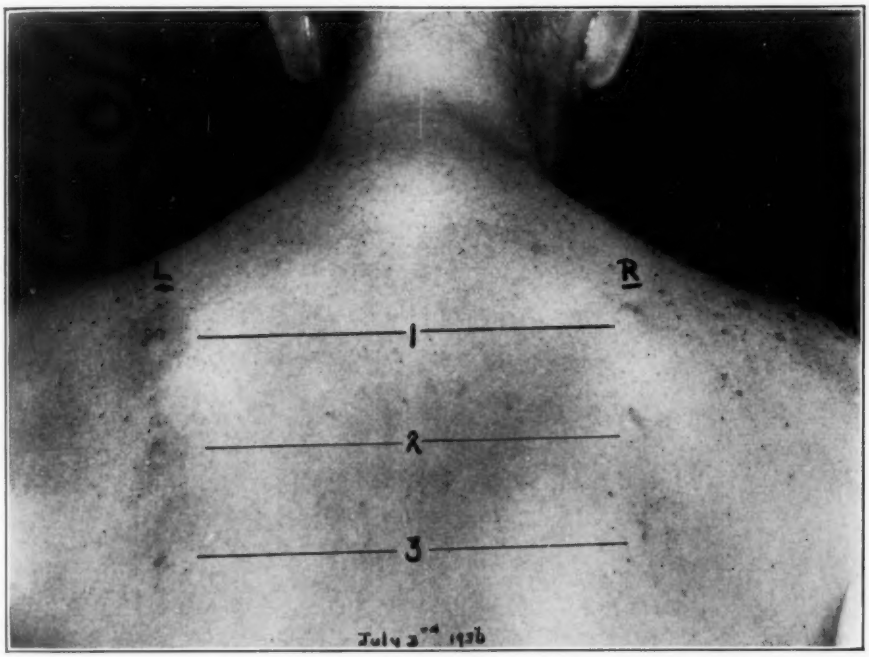

Fig. 1*B*.

TABLE I

PRAUSNITZ-KÜSTNER REACTIONS

Demonstration of Skin Sensitizing Antibodies in Serum From Guinea Pig Experimentally Sensitized and Man Spontaneously Sensitized

July 2, 1936 Sites injected with 0.05 c.c. serum or serum dilutions as noted below.
July 3, 1936 All sites injected with 0.05 c.c. ragweed extract (W. E.) with reactions as noted below and as illustrated in Figs. 1*A* and *B*.

LEFT SIDE			RIGHT SIDE		
SITE		DEGREE OF REACTION	SITE		DEGREE OF REACTION
1	G. P. 503 Undil.	2½-plus	1	G. P. (Nor.) Undil.	Negative
2	G. P. 503 Dil. 1/10	1½-plus	2	G. P. (Nor.) Dil. 1/10	Negative
3	Hu. (Nor.) Undil.	Negative	3	Control W. E. only	Negative
4	Hu. (Nor.) Undil.	*--------	4	*----------	--
5	McL. Undil.	3-plus	5	*----------	--
6	McL. Dil. 1/10	2-plus	6	*----------	--
7	McL. Dil. 1/100	1½-plus	7	*----------	--

Materials used and abbreviations with Recipient B.L.T.

G.P. 503, Serum from guinea pig sensitized with ragweed extract, blood drawn June 6, 1936.

G.P. (Nor.), Serum from normal guinea pig, blood drawn June 16, 1936.

McL, Serum from case of ragweed hay fever.

Hu. (Nor.), Serum from normal human being. Ragweed extract W.E. 2636.

*Not applicable, These sites were injected with other antigens which are without relationship to the results reported upon.

identical titres to that obtained with guinea pig Serum 503. Whether the antibody content of different guinea pigs under similar or varying modes of sensitization will vary as it does with different cases of ragweed hay fever remains to be determined.

In Table II the positive results shown in Table I with guinea pig Serum 503 by means of the Prausnitz-Kustner reaction are repeated, using a different recipient. In this experiment there is also shown the specific and almost com-

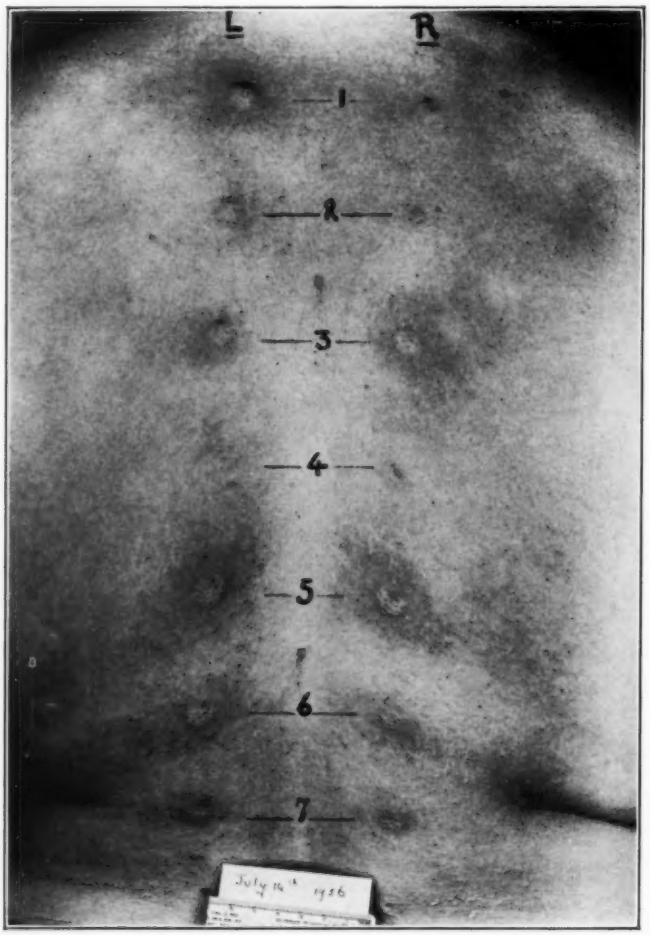

Fig. 2A.

plete absorption of skin sensitizing antibodies from guinea pig Serum 503. This was accomplished by adding antigen (ragweed extract dilution 1/10)* to the antibody (serum of Guinea Pig 503) in the proportion of 1 to 4, and allowing the mixture to stand at room temperature for two hours before injection. As a control, saline was added in the same proportion to the antibody and treated in the same manner. The results are shown in Site 3, left and right, respectively. The reactions obtained with serum of a different case

*By weight of original pollen per volume of extracting fluid this figure actually represents a dilution of 1/200. This same ratio applies to all dilutions of antigen in this paper.

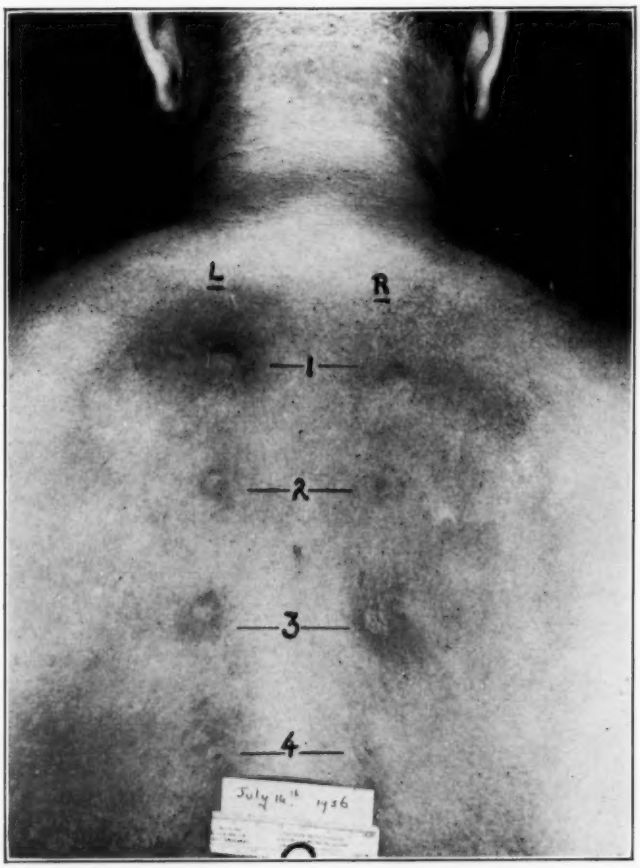

Fig. 2*B*.

TABLE II*

PRAUSNITZ-KÜSTNER REACTIONS

Demonstration of Specific In Vitro Absorption of Antibody in Guinea Pig Serum

July 13, 1936 12 A.M.: 0.2 c.c. serum of Guinea Pig 503 mixed with 0.05 c.c. ragweed extract, labelled G. P. 503 Rag.

12 A.M.: 0.2 c.c. serum of Guinea Pig 503 mixed with 0.05 c.c. saline, labelled G. P. 503 Sal.

July 13, 1936 2 P.M.: Sites injected with 0.05 c.c. serum, serum dilutions, or mixtures as noted below.

July 14, 1936 11:30 A.M.: All sites injected with 0.05 c.c. ragweed extract with reactions as noted below and as illustrated in Figs. 2*A* and *B*.

LEFT SIDE		RIGHT SIDE	
SITE	DEGREE OF REACTION	SITE	DEGREE OF REACTION
1 G. P. 503 Undil.	3-plus	1 G. P. (Nor.) Undil.	Negative
2 G. P. 503 Dil. 1/10	1-plus	2 G. P. (Nor.) Dil. 1/10	Negative
3 G. P. 503 Rag.	½ or ?	3 G. P. 503 Sal.	2½-plus
4 Hu. (Nor.) Undil.	Negative	4 Control	Negative
5 S. Serum Undil.	4-plus	5 S. Serum 1/10	3½-plus
6 S. Serum 1/100	3-plus	6 S. Serum 1/500	2-plus
7 S. Serum 1/1500	1-plus	7 S. Serum 1/5000	½-plus

*Abbreviations used with this Recipient L.A. similar to those in Experiment 1. S. Serum from a case of ragweed hay fever.

of ragweed hay fever are shown under the caption "S. Serum" in Sites 5, 6, and 7, both left and right. With this particular serum the antibody content shows decreasing reactions up to and including the final dilution of 1/5000. The controls, namely, the sites in which normal guinea pig serum and normal human serum were injected, are negative as well as the unprepared or normal Site 4 (left) in which ragweed extract only was injected.

The interpretation of the results of the tests shown in Tables I and II seems obvious. The only objection possible would seem to be that the positive reactions obtained in the sites sensitized with guinea pig Serum 503 were non-

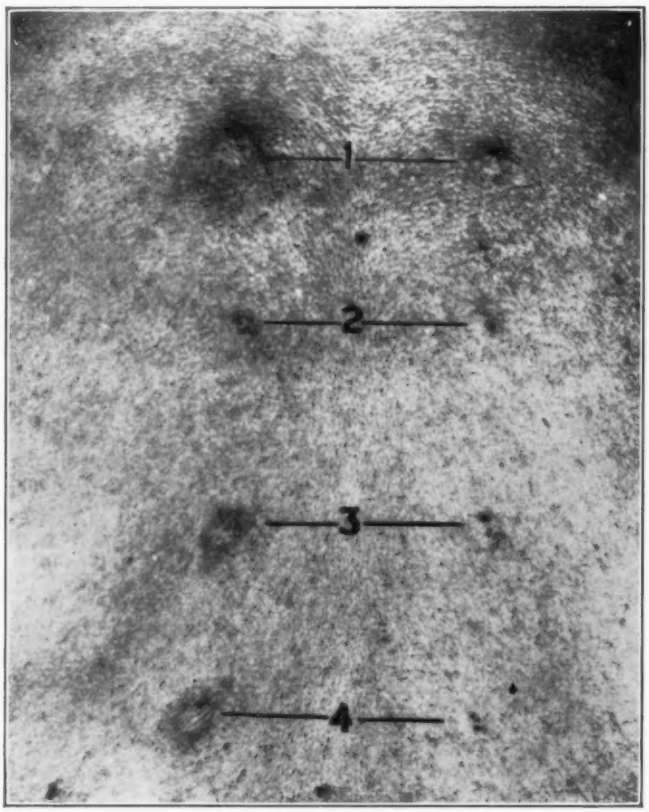

Fig. 3A.

specific. This contention is disproved by the results obtained when two non-related antigens are used as shown in Tables III and IV and Figs. 3A and B.

In this experiment we have used the serum from a human case of ragweed hay fever (Doy.) who, as well, was sensitive to rat hair, i.e., had given positive scratch tests to rat hair and as well had evidence of clinical distress in handling rats in a hospital laboratory. This rat antigen was used as a control in a site sensitized with guinea pig Serum 503 in a third recipient (low). No reaction resulted following the injection of rat hair extract into a site sensitized with guinea pig Serum 503. The rat extract was biologically active as shown by the positive reaction obtained in the human serum sensitized site. A second

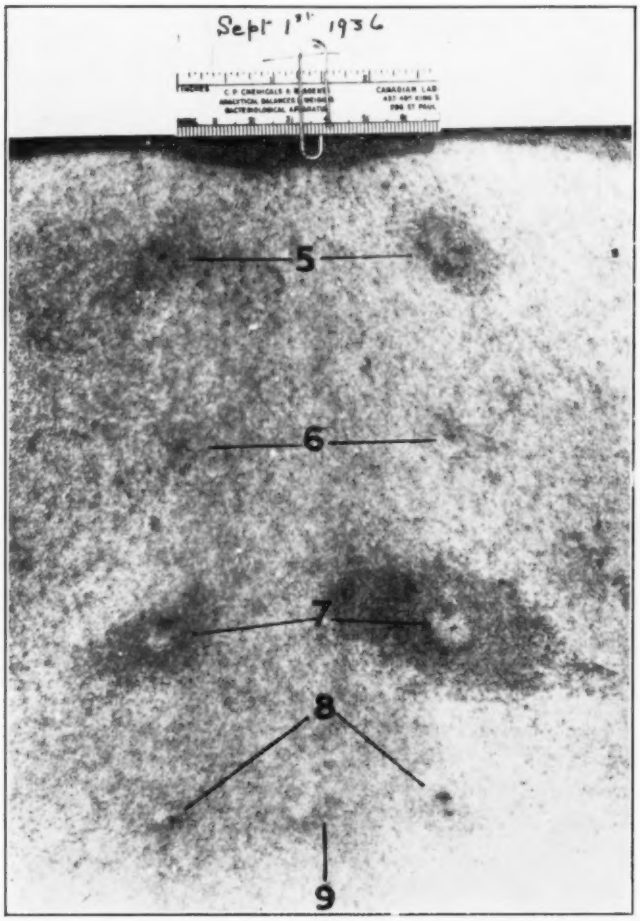

Fig. 3*B*.

TABLE III†

PRAUSNITZ-KÜSTNER REACTIONS

Aug. 31, 1936 Sites injected with 0.05 c.c. serum as noted below.
Sept. 1, 1936 Sites injected with 0.05 c.c. antigen as noted below.

LEFT SIDE				RIGHT SIDE			
SITE		ANTIGEN	REACTION IN MM.	SITE		ANTIGEN	REACTION IN MM.
1	G. P. 503	Ragweed	65 × 45	1	G. P. 503	Rat	Negative
2	G. P. (Nor.)	Ragweed	Negative	2	O Control	Ragweed	Negative
3	G. P. 527	Ragweed	25 × 20	3	G. P. 527	Egg Alb.	Negative
4	*---------	*-------		4	O Control	Egg Alb.	Negative
5	*---------	*-------		5	*-------	*-------	
6	*---------	*-------		6	Hu. Nor.	Ragweed	Negative
7	Hu. Doy.	Rat	30 × 15	7	Hu. Doy.	Ragweed	75 × 40
8	*---------	*-------		8	*-------	*-------	

*Not applicable. These sites were injected with other serums and antigens, and the results have no bearing on the present report. Recipient L. R.

†Site 9.—O Control Rat—Negative.

nonrelated antigen, namely, a solution of egg albumen (diluted to a total nitrogen content equal to that of the ragweed solution used), was injected in a site sensitized with guinea pig Serum 527. This serum was taken from a guinea pig sensitized with alum-precipitated ragweed extract as previously described. In Table III the details given are those which show conclusively that the positive results obtained in human skin sites injected with suitably sensitized guinea pig serums are specific. Cross diameter millimeter measurements of the areas referred to are given in Table III and the numbers given correspond to those used in the illustration.

In Table IV are given the cross millimeter measurements of the reaction obtained on the reinjection of the different antigens as indicated. These results confirm our contention that the reactions obtained are specific.

TABLE IV

PRAUSNITZ-KÜSTNER REACTIONS

Sept. 2, 1936 Sites reinjected with 0.05 c.c. antigen as noted.

LEFT SIDE			RIGHT SIDE		
SITE	ANTIGEN	REACTION IN MM.	SITE	ANTIGEN	REACTION IN MM.
1	Ragweed	Negative	1	Ragweed	30.20
3	Ragweed	Negative (?)	3	Ragweed	12.10 ?
7	Ragweed	70 × 35	7	Rat	12 × 10
8	Ragweed	Negative	8	Ragweed	25 × 15

CONCLUSION

Serum from a guinea pig suitably sensitized with ragweed pollen extract contains human skin sensitizing antibodies that are specific, and would seem to be identical with those present in the serum obtained from man naturally sensitized.

These results were obtained by means of the Prausnitz-Kustner reaction and a standard modification thereof.

A STUDY OF THE HEAT SENSITIVITY OF THE MENINGOCOCCUS IN VITRO WITHIN THE RANGE OF THERAPEUTIC TEMPERATURES*

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THE treatment of certain chronic infectious diseases by artificial fever produced by physical methods has been generally acknowledged as of value and is employed routinely when the indications warrant. This method of treatment is especially efficacious in the various forms, both acute and chronic, of gonorrheal infection, and in gonorrheal arthritis in particular, cures of which have been reported in from 50 to 100 per cent of cases in the series that have appeared in the literature, with complete relief occurring in a total average of 92 per cent.[1] The promptness of response has seemed in many cases spectacular and the treatment is now regarded by many as specific for this form of arthritis. As regards other forms of gonococcal infection many cases of cervicitis, urethritis, and salpingitis, which have not responded to other forms of treatment, have yielded to fever therapy. This excellent clinical result is considered to be due in large measure to the sterilizing effect of heat upon the gonococcus, the thermal death point of which has been shown by Carpenter, Warren, and their associates[2] to lie within a range of heat which can be tolerated by man. However, it has also been suspected that the bacteriolytic effect of heat is not the only factor responsible for these results, for in some instances the Rochester workers report that "cures" resulted when they were unable to obtain in vitro death of the strain for a similar exposure, and they concluded that some injury other than that due to heating occurs in the gonococcus, making it more susceptible to destruction by the normal defenses of the body. Such factors must likewise account for the good results obtained by observers who report a high percentage of "cures" with temperatures lower than that required for the in vitro death of any strains reported by the Rochester workers. Whether these favorable results are due to stimulation of the bactericidal defenses of the body or to other physiologic effects of heat, it is significant that in some cases, at least, the complete eradication of all the organisms by the heat factor alone probably does not occur. The physiologic changes which occur during hyperpyrexia have been widely studied and reported. These have been recently summarized by Hench. Among the most important factors in the treatment of an infectious disease would seem to be the development of a marked leucocytosis in response to heat, and with it "an active phagocytosis."[3] Hench[4] mentions, also, the influence upon the infecting agent of "an indirect bacteriolytic or bacteriostatic effect resulting

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Received for publication, April 30, 1936.

from an increasing formation or mobilization of immune bodies," although the data regarding this factor are meager and contradictory. It is probable that an important physiologic effect of heat in the treatment of an infectious disease is a local vasodilatation "providing an augmented blood supply to inflamed tissues." Finally, also, it has been pointed out that a beneficial effect occurs as a result of the heightened metabolism incident to fever. It is beyond the scope of this paper to offer more than speculation as to the possible application of fever therapy to meningococcus infections. Hazards would undoubtedly be encountered in the treatment of meningitis, in particular, that could not be predicted by a knowledge of the physiologic effects of heat under normal conditions or in other chronic or acute diseases. However, because of the success attending the treatment of infections caused by the gonococcus and the biologic similarity of the meningococcus and the gonococcus, a study of the heat sensitivity of the meningococcus seemed deserving of study.

Many observations have been made on the thermal death time of the meningococcus, but these are concerned primarily with the length of time and degree of heat required to kill the organism without regard to the question of human tolerance. The present study was undertaken to determine the effect of heat upon the growth of this organism within the range of temperatures that have been in general use in the administration of artificial fever.

METHOD

Fifteen strains of meningococci were obtained for this study, all of which were supplied through the courtesy of the Bureau of Laboratories of the New York Department of Health. These strains had been isolated at varying intervals within three months of the period of study, ranging from two days to several months from the time of recovery of the strains. The source was the cerebrospinal fluid in cases of meningococcus meningitis. The cultures were kept on the semisolid veal infusion agar described by Miss Ruth Gosling[5] of the Department of Applied Therapy of the Bureau of Laboratories. An attempt was made to adapt the method used by Carpenter and others[6] in their study of the thermal death time of the gonococcus which consisted in exposing the cultures growing in a fluid medium to heat in a water-bath, with subsequent dilution and plating upon a solid medium. However, this method did not prove satisfactory for the meningococcus as the final growth in the solid medium produced inconsistent results. A method was used, therefore, that did not require adaptation of the growth from a liquid to a solid medium, and in which there proved to be consistent and uniform growth in the controls. This medium was the same as that on which the stock culture had been maintained—the semisolid veal infusion agar described by Gosling and referred to above, except that it was enriched by ascitic fluid (50 per cent) and the agar content increased from 0.25 to 1 per cent for purposes of plating. The reaction was adjusted so that after the addition of ascitic fluid the final reaction pH was 7.6. The strains to be studied were diluted as described below and pour plates made, after which the plates were exposed to the desired tempera-

tures. The meningococcus grew well in this medium when plated; moisture in the plates was conserved by sealing with adhesive tape, and humidity in the incubator was secured by water in open containers.*

The procedure of the experiment follows: A 2 mm. loop of the strain, a twenty-four-hour growth upon the semisolid medium, was emulsified in 10 c.c. of normal salt solution, thoroughly shaken and a further dilution made (1:10). One cubic centimeter of the dilution was then transferred to each of a series of test tubes containing 5 c.c. of ascitic fluid. The seeded ascitic fluid was then poured into tubes containing 6 c.c. of 2 per cent agar dextrose veal infusion medium, thus reducing the agar content to 1 per cent which proved to be the minimum concentration requirement for plating. The reaction of the agar medium had been previously adjusted, so that after the addition of ascitic fluid the final reaction was 7.6 pH. The 2 per cent agar medium was cooled before the seeded ascitic fluid was added to 42° C. in the water-bath, and the rapid cooling of the medium by the addition of an equal amount of ascitic fluid at room temperature made it necessary to pour the plates at once to prevent jelling in the tube. The plates were sealed with adhesive tape and placed in the incubators, the control plates in the incubator set at a temperature of between 36° and 37° C., and the remainder in the incubator set at a temperature, the effect of which was to be studied. At the desired interval two plates were transferred from the high to the lower (control) temperature incubator, and all plates were examined for the number of colonies present after twenty-four, thirty-six, and forty-eight hours. A satisfactory colony count could be made as a rule after twenty-four hours. From three to ten determinations were made on each strain. As a result of unavoidable fluctuations within the incubators, the temperature in each incubator varied from 0.4° to 1° C. The amount in each instance was noted and recorded. Although unavoidable, this fluctuation did not seem entirely undesirable, since some variation in body temperature occurs during fever therapy, and growth under these conditions might therefore appear more representative of in vivo conditions. The temperatures accordingly lay within limited ranges between 40° and 42° C., the average fluctuation not exceeding 0.4° C., which was noted for each determination of the several used for each strain studied. In a few instances a temperature as low as 39° C. and as high as 42.4° C. was included, although the latter temperature probably could not be used for therapeutic purposes.

RESULTS

The growth of all strains of meningococci studied, with a few exceptions, was either destroyed or reduced in periods of time varying from three to seven hours, which would indicate its clinical applicability. Intervals thereafter ranging from seven hours to as long as three days or longer were also observed at temperatures of from 40° to 42° C. Each strain was, in general, consistent in its response to heat, but the strains varied in the length of exposure and the degree of heat necessary to destroy them or markedly to

*I wish to express my appreciation to Dr. John C. Torrey for his suggestions as to the cultivation of the meningococcus.

reduce their growth. Some strains were consistently destroyed at lower temperatures and with shorter exposures than others which seemed relatively heat resistant. Of the fifteen strains studied, five were definitely heat sensitive, seven were moderately heat sensitive, two strains were slightly affected, and one proved markedly heat resistant at the temperatures to which they were exposed. The heat sensitivity of the strains apparently did not depend upon

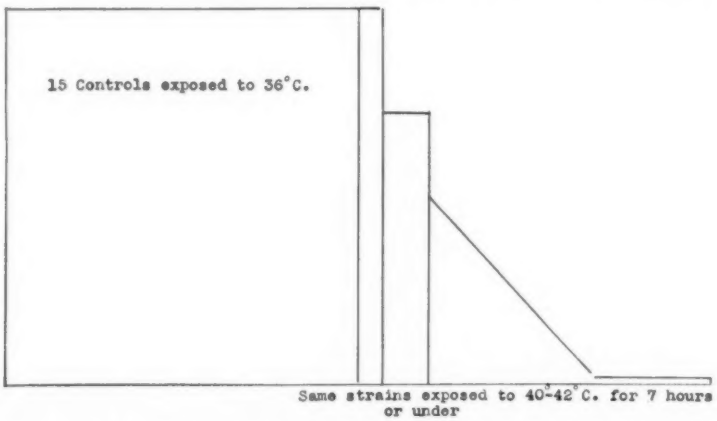

Fig. 1.—Schematic summary of effect of therapeutic temperatures upon fifteen strains of meningococci. Five strains destroyed in seven hours, 7 strains markedly reduced, 2 strains slightly affected, and 1 strain not reduced.

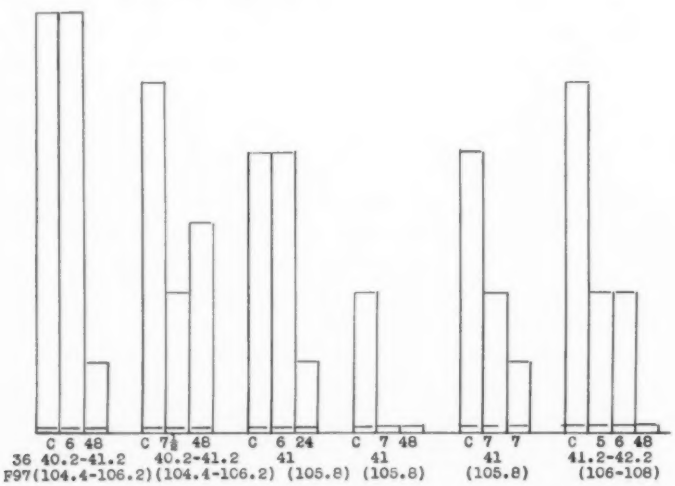

Fig. 2.—Heat sensitive (Strain No. 25-422). At 40.2° to 41.2° C, (104° to 106° F.) growth reduced in six to seven hours. One plate survived forty-eight hours at this temperature. In five hours at 41.2° to 42.2° C. (106° to 108° F.) growth three-fifths destroyed—entirely in forty-eight hours.

the length of time that they had been cultivated upon artificial media, as there was no observed relationship between the heat sensitivity and the interval since isolation. However, all cultures were relatively recent (maximum age three months).

It was noted that plates exposed to an equal amount of heat occasionally varied in the number of resulting colonies. This was thought to be due to an

unequal distribution within the strain of heat sensitive and heat resistant units. The unequal distribution of these units would seem to account for the fact that in all of the strains, which were uniformly destroyed or markedly reduced by heat, an occasional colony or scattered growth of colonies survived on the plates, although the temperature was sufficient to destroy the majority

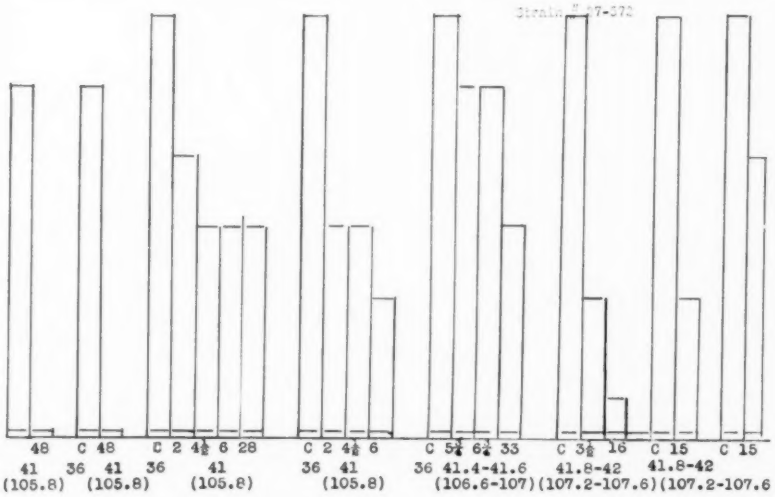

Fig. 3.—Heat sensitive (Strain No. 27-572). Growth reduced in two to six hours at 41° C. (105.8° F.). Growth destroyed in three and one-half hours at 41.8° to 42° C. (107.2° to 107.6° F.).

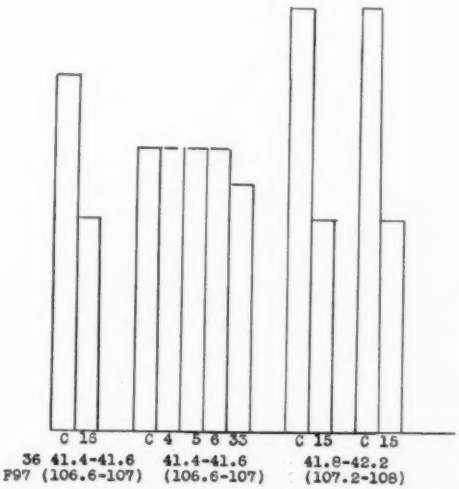

Fig. 4.—Heat resistant (Strain No. 27-574). No reduction in six hours or under at 41.4° to 41.6° C. (106.6° to 107° F.); in fifteen to thirty-three hours, slight reduction. Reduction occurred in fifteen hours at 42° C.

of organisms in the strain. The strains that contained the largest number of such surviving colonies were classified as heat resistant. As stated, an unequal distribution of such units in the strains appeared to account for the survival of some colonies on plates that had been exposed to higher temperatures and for longer periods of time than others in which no growth occurred.

Although the time period chosen for routine exposure was five hours, seven hours was occasionally used and several strains were exposed for shorter intervals. Of the three strains examined at a two-hour interval, two showed marked reduction in growth (No. 27-572; No. 26-016). Two strains examined at three hours and three and one-half hours showed reduction of growth (No. 27-534; No. 27-582). The average interval used for exposure of the plates of five to seven hours was chosen as corresponding to the customary duration of sessions of artificial fever. At this interval, therefore, the following results were obtained: Marked reduction occurred in seven strains

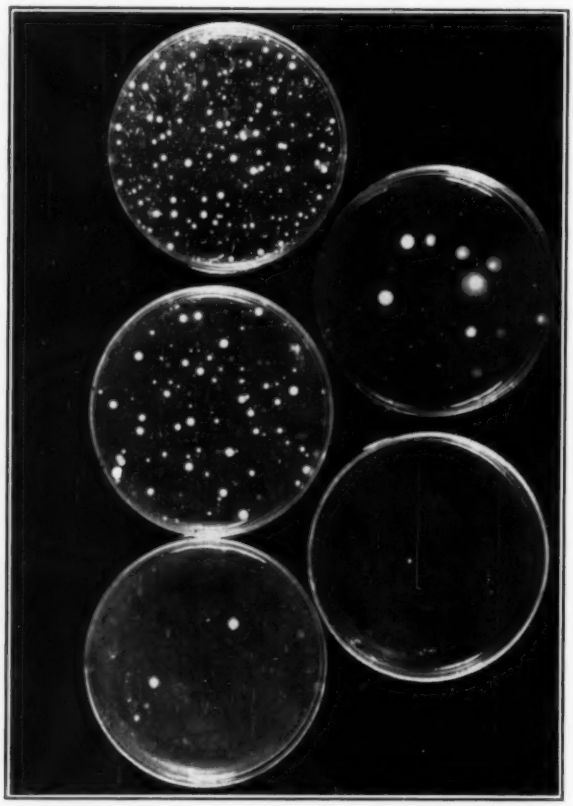

Fig. 5.—Strain No. 26-016. Control plate incubated at 36° to 37° C. Plates exposed at a temperature of from 41.6° to 41.8° C. for two, three, four, and five hours.

(No. 26-221; No. 26-247; No. 25-422; No. 26-048; No. 26-158; No. 26-627; No. 25-943). Destruction occurred in two strains (No. 26-430; No. 27-582). In two strains the growth was very little affected in five hours. In one (No. 27-574) no perceptible reduction occurred in five hours. Although there was reduction of growth in two strains in as early as the two- and three-hour interval, destruction occurred in four or five hours. In other strains although an early reduction occurred, more prolonged exposure did not invariably affect the amount of growth which was finally destroyed, if at all, in from sixteen hours to three days. In one strain, indefinite heating at these temperatures (six days) did not cause complete suppression. Whether heating had a dele-

terious effect upon the surviving organisms, however, cannot be stated. Neither does the method used for the experiment provide any evidence as to the optimal growth temperature for the meningococcus. It is possible that growth may be stimulated by temperatures slightly above the body temperature and that obvious danger would exist in exposing patients to heat slightly above the body temperature but under that at which the growth depressing effect became operative. According to the report of the Medical Research Council,[7] the optimal growth is accepted as to occur between 36° and 37° C. Dopter,[8] however, claims the optimal growth temperature to be 38° C. In the present study, although 39° C. was occasionally used, in no plate was there

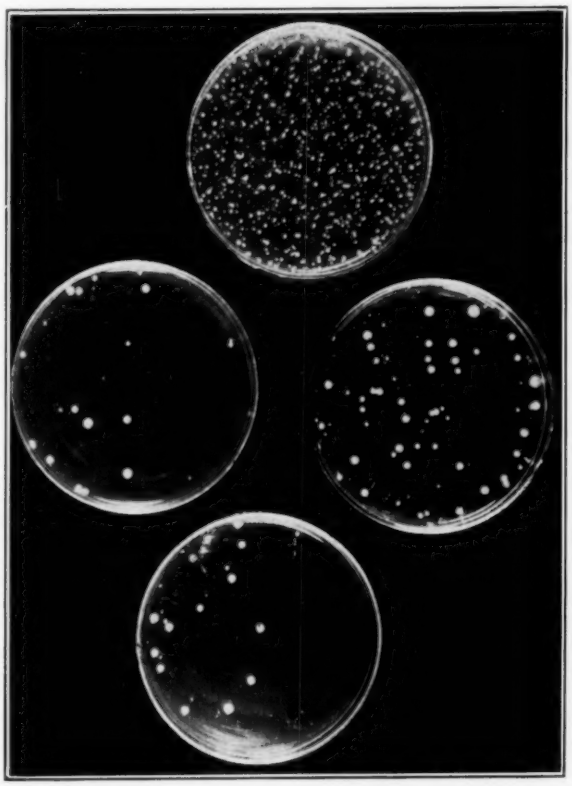

Fig. 6.—Strain No. 26-221. Control plate incubated at 36° to 37° C. Plates exposed at a temperature of from 41.2° to 42.2° C. for five hours (two plates) and twenty-four hours (one plate). Note: Twenty-four-hour plate contains approximately the same number of colonies as one of the five-hour plates. Heat sensitive units destroyed within the first five hours.

a larger number of colonies than in the controls incubated at 36° to 37° C. At temperatures of 40° to 42° C. all of the strains except one showed reduction of growth in plates exposed for five hours. In some strains a growth depressing effect did not occur until the lower point of the temperature fluctuation was elevated above 41° C. This is illustrated by strain No. 27-534 in which no effect was noticed with the lower point of the fluctuation at 41° C. At 41.2° C., however, definite reduction occurred. This strain brings to mind the statement made by Dopter (1921) that "at 41° C., though so close to optimal, the organisms die." It seems evident from this experiment, however,

TABLE I

ESTIMATED AMOUNT OF GROWTH IN PLATES EXPOSED FOR STATED PERIODS OF TIME TO THERAPEUTIC TEMPERATURES

STRAIN NO.	40°-41° C.		41°-42° C.	
	UNDER SEVEN HOURS	OVER SEVEN HOURS	UNDER SEVEN HOURS	OVER SEVEN HOURS
Heat Sensitive Strains				
26-016	Destroyed in 5 hours (2 plates)	Destroyed in all hours over 5 (3 plates)	Destroyed or greatly reduced in 3, 4, and 5 hours (8 plates) ⅓ reduced in 2 hours	Destroyed in all hours
25-422	Plates showed ½ to ¾ reduction (3 plates); complete destruction (1 plate) in 7 hours	½ to complete destruction in 24 and 48 hours (3 plates)	¾ reduction in 5 and 6 hours (4 plates)	¾ reduction in 24 hours (1 plate) Complete destruction in 48 hours (1 plate)
27-572	⅓ reduced in 2 hours (2 plates) ½ reduced in 4½ to 6 hours (4 plates)	½ reduced in 28 hours (1 plate) Destroyed in 48 hours (2 plates)	¼ to ¾ reduced in 3½ to 7 hours (3 plates)	½ to complete destruction in 15 to 33 hours (4 plates)
26-430	Destroyed in 5 hours (2 plates)	Some growth present in 24-hour plate (1)	Destroyed in 5 hours (4 plates)	Destroyed in all hours (2 plates)
27-582	Not examined at this temperature		Destroyed in 5 hours (4 plates) Reduced ¼ to ½ in 2 and 3 hours (2 plates)	Destroyed in all hours (16 and 26) (2 plates)
Heat Resistant Strains				
25-404	No reduction in 6 hours (1 plate) ⅓ reduced in 7½ hours (2 plates)	No reduction in 24 hours (1 plate) Slight reduction in 48- and 72-hour plates (2)	¼ reduction in 5, 6, and 7 hours (6 plates)	No further reduction in 24 and 48 hours (3 plates)
27-574	Not examined at this temperature		No reduction in 4, 5, and 6 hours (3 plates)	½ reduced in 15 hours; no further in 33 hours (3 plates)
27-570		⅗ reduced (1 plate); complete destruction (1 plate) in 48 hours	¼ reduced in 5 and 6 hours (2 plates)	¼ to ½ reduced in 24 to 26 hours (2 plates) Destroyed in 24 to 26 hours (2 plates)

TABLE I—CONT'D

STRAIN NO.	40°-41° C.		41°-42° C.	
	UNDER SEVEN HOURS	OVER SEVEN HOURS	UNDER SEVEN HOURS	OVER SEVEN HOURS
	Moderately Heat Sensitive Strains			
26-158	½ reduced in 5 hours (2 plates)	½ reduced in 24 hours	½ reduced in 5 hours (2 plates) Destroyed in 5 hours (2 plates)	Destroyed in 24 hours (2 plates)
26-627	½ reduced in 5 hours (2 plates)	Completely destroyed in 24 hours (1 plate)	No reduction (1 plate) in 5 hours ½ reduced (1 plate) in 5 hours Completely destroyed in 5 hours (2 plates)	Destroyed in 24 hours (2 plates)
25-943	Destroyed in 7½ hours (1 plate)	Destroyed in 18, 24, and 48 hours (3 plates) (a few colonies on two plates)	¼ to ¾ reduced in 5 hours (4 plates)	¾ reduced in 7, 48 hours and some colonies present in 6 days
26-221	¼ reduced in 5 hours (2 plates)	Destroyed in 24 hours	½ to ¾ reduced in 5 hours (4 plates)	A few colonies present in 24 hours (2 plates)
26-247	¼ reduced (1 plate); no reduction 5 hours (1 plate)	¾ reduced in 24 hours (1 plate)	¼ to ¾ reduced in 5 hours (6 plates)	Destroyed in 24 hours (2 plates) Some growth present in 33 hours (1 plate)
26-048	½ to ⅘ reduced in 5 hours (2 plates)	⅘ reduced in 24 hours (1 plate)	½ to ⅘ reduced in 5 hours (6 plates)	Destroyed in 24 hours (2 plates) Some growth present in 24 hours (1 plate)
27-534	Not examined	No reduction 48 hours (2 plates)	¼ to ¾ reduced in 3½, 4¾ and 5¾ hours (3 plates)	½ to 5/6 reduced in 15 and 16 hours (3 plates)

Note on terms used:
"Destruction": No colonies or less than five on exposed plates.
"Reduction": Number of colonies as compared with control grown at 36° to 37° C.

that no such general statement may be made. The practical significance of the observation on this strain is also apparent. The therapeutic temperature accepted as most satisfactory in the treatment of gonorrhea is considered to be not less than 41.6° C., and this temperature from a theoretical standpoint would seem the desirable level should a clinical application to the treatment of meningococcus infection be attempted.

SPECULATION AS TO THE POSSIBLE CLINICAL APPLICATION OF THIS STUDY

As regards the possible use of fever therapy in meningococcus meningitis and its complications, it is conceded that it would be impossible to predict whether a patient so acutely ill as the average patient with meningitis would tolerate temperatures higher than naturally occur in this disease. If so, the question arises as to whether we are justified in assuming that the bactericidal effect of heat would be the same in vivo as in vitro. The reply of the Rochester observers as pertains to the gonococcus may be quoted: "The abnormal environment resulting from the artificial cultivation cannot be more injurious to the gonococcus than the natural defense agents other than fever." Some of these agents, it will be recalled, were mentioned in the introductory discussion of fever therapy as the result of the physiologic effect of heat upon the cellular elements of the blood, the mobilization of immune bodies, the local vasodilatation and augmented blood supply to inflamed tissue and the general effect from heightened metabolism incident to fever. As to the local effect upon the specific disease process, those workers who have used hyperpyrexia in the treatment of pelvic inflammatory disease have observed the early resolution of exudate and fibrin and the prevention of adhesions as the result of this absorption. For this purpose local heat as is secured by the use of the Elliott machine has been advocated by certain gynecologic clinics as a postoperative measure following plastic operations on the fallopian tubes, to hasten the absorption of exudate and fibrin and so prevent the formation of adhesions. Arguing from this analogy it is possible that hyperpyrexia would likewise hasten the absorption of meningeal exudate and so prevent the development of adhesions and the resulting block to the circulation of cerebrospinal fluid, a dread complication of the disease. Although the treatment of meningococcus meningitis with specific serum is highly effective, it is possible that hyperpyrexia would prove of advantage as a supplementary measure in those cases that have resisted serum therapy or in which serum for some reason may be contraindicated, or when block has occurred as an inaccessible barrier to the administration of serum, or in cases in which other complications arise, as for example, in the joints. Herrick and Parkhurst[9] state that meningococcus arthritis occurs in from 4 to 7 per cent of cases of meningococcus meningitis. Since hyperpyrexia is so effective in gonorrheal arthritis as to be regarded as specific for this condition, it seems conceivable that it might prove equally effective for meningococcic arthritis. To stress further this analogy between meningococcic infection and gonorrheal arthritis, the latter is an infection present in a closed serous cavity. Should the meningococcus prove to be as heat sensitive in vivo as the gonococcus and since meningitis also occurs in a closed system bathed in serous fluid, would it not be permis-

sible to expect a similar destructive effect upon the causative agent? The argument from such an analogy, however, naturally fails in the consideration of the presence of vital centers, with the possibility of injury that may have occurred to them as a result of extension of the inflammatory process from the meninges and which the added demands incident to fever might further jeopardize. The possible danger of a localized increase in pressure as a result of hydremia of the tissues or of an increased production of cerebrospinal fluid must also be considered. It may be pointed out, however, that hyperpyrexia is attended by a depletion of fluid within the body and that this dehydration would quite possibly be sufficient to counteract any such tendency to localized hydremia. That the method is relatively safe under controlled conditions and in the diseases for which it has been used is borne out by the report of the Council of Physical Therapy of the American Medical Association[10] in which is listed 29 deaths among 4,809 patients who had various diseases, a mortality rate of 0.6 per cent. Hench and his co-authors[11] in commenting on this report point out that many of these deaths were undoubtedly of patients who had neurosyphilis, and that some deaths should be ascribed to the disease and not to the treatment. Although this statement is not supported by statistical data and although the assumption may reasonably be justified, it may not necessarily be anticipated that parenchymal damage to the central nervous system would prove a definite contraindication to the treatment or that a high mortality rate need inevitably be expected in such cases. In neither neurosyphilis nor in chorea has the reported mortality rate been remarkable. The possibility, therefore, of a spread of meningeal infection to adjacent brain tissue need not be considered with undue apprehension as a contraindication to fever therapy. In general, furthermore, meningitis is essentially a disease of the meninges. Certain of the deaths reported in the literature have occurred in patients depleted by chronic illness. In an acute disease such as meningitis the patient would seem reasonably better able to adapt himself to environmental changes than the patient whose vital forces had been spent by a chronic disease. It is with considerable misgiving that the foregoing arguments for the treatment of meningitis by artificial fever are presented, for experience may prove them to be specious and misleading. If such treatment is ventured it should be attempted only in the carefully selected case, in which other methods of treatment have failed, under conditions which are carefully controlled and with a full appreciation of the risk that must be accepted in the individual case.

SUMMARY AND CONCLUSIONS

1. The heat sensitivity of fifteen strains of meningococci in vitro was determined at fever temperatures ranging between 40° and 42° C.

2. All strains except one showed reduction of growth with five hours or within seven hours of exposure to these temperatures.

3. At 39° C. there was no apparent effect upon the growth. The maximum reduction occurred at temperatures of 41° C. and above.

4. Five strains were definitely heat sensitive, either destroyed or greatly reduced in five hours or less; seven strains showed moderate reduction, the number of colonies on exposed plates reduced by one-half to three-fourths of

those grown at body temperature in seven hours or under. Two showed slight reduction of growth and one was apparently not affected within seven hours at temperatures between 40° and 42° C.

5. The variable heat sensitivity of the strains as well as the presence of some apparently heat resistant units in the majority of strains makes it seem unlikely that a sterilizing effect upon the meningococcus could be obtained in one fever session as a result of the heat factor alone.

6. Fever therapy seems worthy of trial in meningococcus meningitis as an adjunct to other treatment in the selected case in which serum therapy has failed.

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UREA AND CHLORIDES IN HUMAN PAROTID SALIVA

Changes at Different Rates of Secretion as Affected by Atropine and Pilocarpine

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THESE experiments were done to determine the effect of atropine and pilocarpine on the concentrations of two of the constituents of the parotid saliva, and to compare these with the concentrations in the blood taken simultaneously.

There is considerable variation between reported analyses of both mixed and parotid saliva;[1-4] in most of these the rate of secretion has not been regarded as an important element. Some of the reported chloride analyses are listed below.

	MG. CL PER 100 C.C.
Hubbell,[2] mixed human saliva	44.9
De Beer and Wilson,[1] dog's parotid saliva	27.9
Clark and Shell,[1] human mixed saliva	50.0
Frerichs,[6] human mixed saliva	40.0
Werther,[5] dog's parotid saliva	12.0 to 36

In the reports above only Werther considers the chloride concentration as a function of the rate of secretion. From some of the data on mixed saliva no good correlation could be made between chloride content and the rate of secretion.

Urea determinations in saliva are reported by Barnett,[7] Hench and Aldrich,[8] Schmitz,[9] and Updegraff.[4] The urea content of mixed saliva averages about 80 per cent of that of the blood; in parotid saliva the urea concentration was found to approach that of the blood at slow rates of secretion.[7]

Ewing[10] analyzed saliva after administration of atropine and pilocarpine finding with atropine a decrease in concentration of organic solids with decrease of rate; a slight increase of per cent of organic solids after pilocarpine.

Chlorides and urea were selected for these tests as the constituents to be followed because accurate methods are available for their determination, and because it was thought that these two might at different rates vary so as to represent different mechanisms in the production of the parotid secretion.

METHOD

Human parotid saliva was collected by the use of a small cup held firmly over the mouth of the parotid duct (by a suction ring), the saliva being allowed to flow into the collecting vessel under its own pressure. For stimulation of the salivary secretion, paraffin was used for the slow, and lemon for the rapid samples. Between the collection of each set of specimens at least 2 c.c. of saliva was discarded to allow removal of the saliva produced by the previous stimulant; this was found to be sufficient to give consistent results. The slow samples showed approximately the same composition when collected either before or after the rapid ones. The atropine and pilocarpine were given by mouth using

doses of atropine sulphate, 1/50 gr., and of pilocarpine hydrochloride, 1/6 gr., and specimens were collected beginning one hour after taking the drugs.

Chlorides were determined by the method of Van Slyke and Sendroy;[11] urea by the method of Van Slyke and Cullen.[11] All determinations were made in duplicate. Blood samples were taken at the same time as the saliva, and the methods for analysis were those cited above.

DISCUSSION

Results of analyses are shown in Table I opposite the blood values. They show a salivary urea content closely approaching that of the blood in some samples, but a chloride concentration much lower than the simultaneously collected blood.

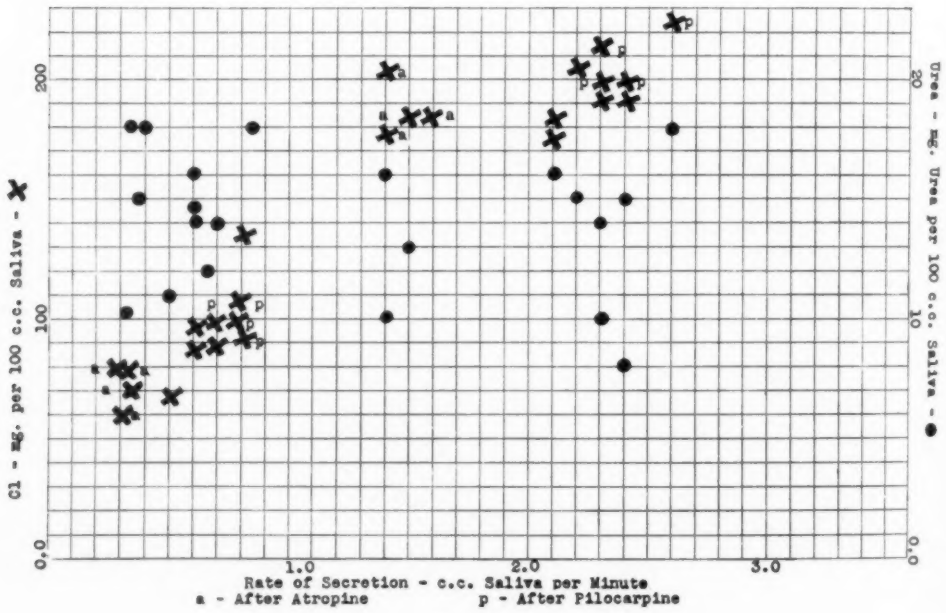

Chart 1.

Chart 1 shows the relation of urea and chloride concentrations to rate of secretion. It is seen that the salivary urea concentration tends to decrease slightly with increase of rate of secretion, although there is considerable variation. In contrast the chloride content of the saliva shows a consistent and marked increase of concentration with more rapid secretion, the saliva produced at high rates having about three times the chloride concentration of that at lower ones. The chart also shows that while the samples collected after pilocarpine have a larger chloride content, this is no more than might be expected from the increased rate of secretion resulting from the drug. Similarly, the low chloride values after atropine are explicable as being merely the result of the decrease in rate.

The difference between the behavior of urea and chlorides with changes of rate of salivary production suggest that the two substances may be secreted

by different paths. If urea were a simple filtrate from the capillaries, one might expect that it would appear in the saliva at a concentration near that of the blood and perhaps drop in concentration with augmentation of salivary secretion. Also, if substances like chlorides were actively handled by the gland cells, one might expect their concentrations in the secretion to be altered by autonomic drugs and possibly by changes in the rate of secretion. These things have been found to occur. The variation of chloride concentration has been known for some time, and together with the results of these experiments several

TABLE I

CHLORIDE AND UREA IN SALIVA AT TWO RATES OF SECRETION COMPARED WITH BLOOD COLLECTED SIMULTANEOUSLY

TEST	PAROTID SALIVA			BLOOD		
	RATE OF SECRETION C.C. PER MIN.	CHLORIDE MG. PER 100 C.C.	UREA MG. PER 100 C.C.	CHLORIDE MG. PER 100 C.C.	UREA MG. PER 100 C.C.	
1	0.8	134	–	378	–	
	2.3	192	–			
2	0.65	99	–	370	15	
	2.1	181	–			
3	2.2	206	15	363	22	
	0.6	87	16			
4	2.4	195	8	378	13	
	0.5	68	11			
5	0.6	96	14	373	19	
	2.1	179	16			
6	0.28	60	15	375	18	After atropine
	1.5	186	13			
7	0.31	70	11	370	15	After atropine
	1.6	187	7			
8	0.27	80	18	367	19	After atropine
	1.4	208	10			
9	0.3	71	18	372	12	After atropine
	1.4	179	16			
10	0.7	99	14	364	17	After pilocarpine
	2.3	217	14			
11	2.6	222	18	366	14	After pilocarpine
	0.6	90	14			
12	0.66	95	12	371	26	After pilocarpine
	2.4	197	15			
13	0.78	106	18	371	14	After pilocarpine
	2.3	197	10			

questions are suggested. One of these is that of the isosmolarity of some body fluids noted by Gamble.[12] Such marked changes of electrolyte content with variation of rate of secretion as occur in saliva would necessitate correspondingly large reciprocal changes in other constituents if isotonicity with plasma is to be preserved. No such findings have been made with saliva, and investigation of this problem might be of some interest.

SUMMARY

Atropine and pilocarpine alter the concentration of chlorides in human parotid saliva only in proportion to the change in rate produced, there being a progressive increase of chloride concentration with increased rate of secretion.

These two drugs cause little change in the urea concentration of the saliva, nor does the rate of secretion.

From the behavior of urea and chlorides at different rates of salivary secretion, it is inferred that urea may be passed through by some process of filtration, whereas chlorides are secreted through the activity of the gland cells.

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223 WEST RAMSEY

PAPILLOMAS OF THE BREAST*

Study of 273 Specimens

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THIS work was undertaken with the following points in mind: first, to determine the incidence of malignant change in papilloma of the breast; second, to test, in a large series of specimens, the views expressed by MacCarty[3-6] in his various publications on the differentiation of malignant cells; and third, to review MacCarty's system of nomenclature of neoplasms with a view of applying his classification to this group of lesions.

In 1923, MacCarty[2] said: "Fresh living cells of malignant neoplasms are perfect spheroidal or ovoidal bodies presenting large spherical or oval nuclei in which may be found one or more large spheroidal or ovoidal nucleoli."

This is a somewhat new conception and introduces the use of absolutely fresh specimens which have been removed surgically. In the following years he and his students restated this first general observation on several occasions. To him, every cell in a tissue may assume one of four forms: an adult form, a regenerative form, an early neoplastic or premigratory form, and a malignant or migratory form (Fig. 1). The last three forms arise in most tissues from the reserve, partially differentiated cells, rather than from the adult or highly differentiated and specialized cells.

The nucleonucleolar ratio has been computed for the cells of many different tissues. This has resulted in the conclusion that the greater the malignancy, the lower the nucleonucleolar ratio. The nucleoli are, in general, much larger in malignant cells[10, 11] than they are in nonmalignant cells. These observations, which originally were made by MacCarty, have been confirmed by Haumeder,[1] Raaf,[15] McCormack,[12] Naidu,[13] and Strohl[16] in America and by Quensel[14] in Europe.

A similar method of approach to that used by these authors has been used in this study. This will be explained more in detail in the consideration of methods.

CLASSIFICATION

Because of the multiplicity and variety of terms by which papillomas of the breast have been classified, it might be well to consider the newer nomenclature. In the literature one finds such terms as "papillary cystome," "villous papilloma," "duct papilloma," "polycytoma proliferum," "proliferous cyst," "epithelioma, intracanalicular cystoma mammae," "papilliferum epithelioma dendritique," "proliferating cyst," "duct cancer," "papillary cystadenoma," "cystadenoma papillare," and many others which are equally confusing and often meaningless.

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Received for publication, July 1, 1936.

In 1932, MacCarty,[7, 8] while considering the nomenclature of cancer and related conditions, said: "I have collected from the literature of the last thirty years more than 950 terms applied to neoplastic conditions." Following this statement, he outlined briefly a simplified classification of neoplasms. His grouping is as follows: (1) those composed of adult cells with normal arrange-

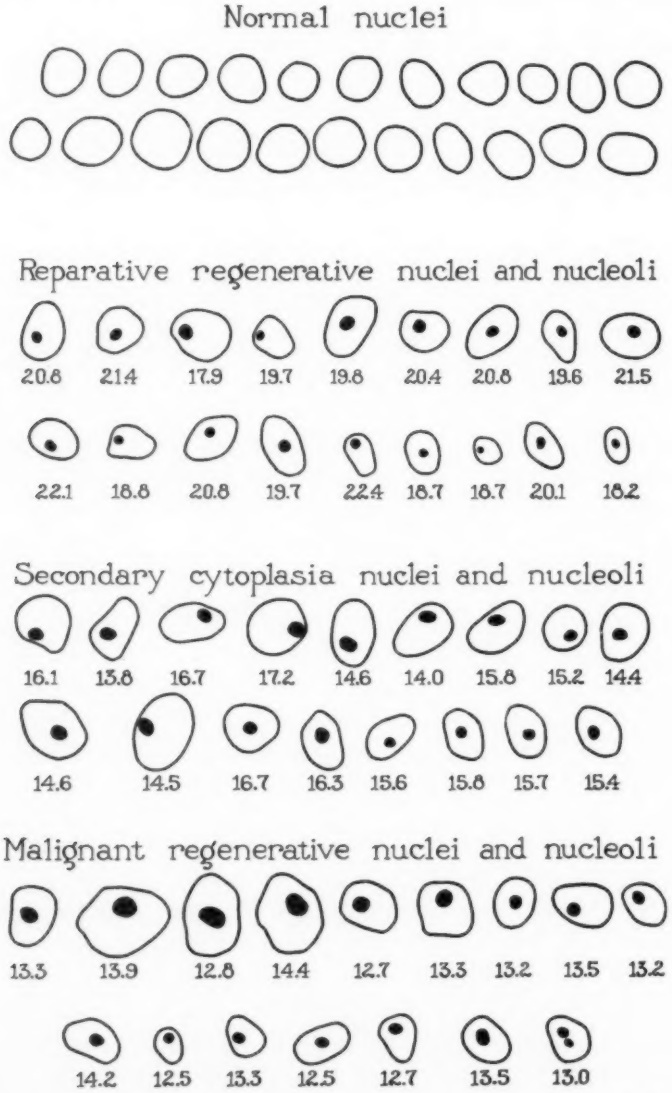

Fig. 1.—Outlines of nuclei and nucleoli in each of the four phases of cellular differentiation; the nucleonucleolar ratios are noted.

ment of tissue (textocytomas), (2) those composed of cells which are arranged normally but have morphology of malignant regenerative cells (textoblastomas), and (3) those composed of cells of the malignant regenerative type which are not arranged in any fashion approaching that of any normal tissue (protextoblastomas).

The first group includes those neoplasms which are clinically benign, while the second and third groups include those which are clinically malignant. He further explained this classification by saying: "Since there are many types of tissues in the body, each group, especially the first two, embraces tumors composed of cells which belong to and simulate histologically one or more of the normal tissues."

To the adult cells which are characteristic of the first group of tumors he gave the name of "textocytes." The tumors of this group consist of the following cells: adenocytes, chondrocytes, endotheliocytes, epitheliocytes, fibrocytes, gliocytes, lipocytes, lymphocytes, melanocytes, myocytes, myxocytes, neurocytes, odontocytes, and osteocytes.

The tumors which consist of these types of cells he has called "textocytomas" or "cytomas."

Tumors of his second group were classified as "textoblastomas" or "blastomas." This group of tumors includes the following types of cells: adenoblasts, chondroblasts, endothelioblasts, epithelioblasts, erythroblasts,* fibroblasts, glioblasts, leucoblasts,† lipoblasts, lymphoblasts,‡ melanoblasts, myoblasts, myxoblasts, neuroblasts, odontoblasts, and osteoblasts.

The third group consists of those neoplasms which are so primitive that there is no suggestive resemblance to any normal adult tissue. MacCarty has called this group of tumors "protextoblastomas" or "problastomas," and since they do not resemble normal tissues, no modifying prefix can be added.

The tumors of the first group, the cytomas, are clinically benign, while the tumors which comprise the second and third groups are clinically malignant. According to this simplified method of nomenclature, described by MacCarty in 1932, the intracystic and intraductal papillomas of the breast might be classed as papillary adenocytomas (benign) or adenoblastomas (malignant), depending on the cellular type shown on microscopic examination. This classification will be used in this paper.

PAPILLARY ADENOCYTOMAS AND PAPILLARY ADENOBLASTOMAS

Papillary neoplasms of the breast occur as intracystic or as intraductal growths from the epithelial lining and the underlying connective tissue stroma. According to MacCarty,[9] this form of growth is observed in about 3.3 per cent of all mammary lesions, which would make it a comparatively common lesion.

Both intracystic and intraductal neoplasms may form a round, movable tumor beneath the nipple or in any portion of the organ (Figs. 2 and 3). They usually grow slowly and may or may not be accompanied by a serous or bloody discharge.

Microscopically, these neoplasms appear as papillary growths attached to one segment of the wall of the duct. Increase in size takes place by growth of the papillary projections. While many of these tumors appear benign, some obviously are malignant.

*Found in pernicious anemia.
†Found in myelogenous leucemia.
‡Found in lymphatic leucemia, lymphosarcoma, and Hodgkin's disease.

METHODS OF STUDY

Two hundred seventy-three breasts were used for this study. These breasts were removed surgically because they contained papillomas. The specimens were divided into two parts: those which were studied in a perfectly fresh, unfixed condition and stained with Unna's polychrome methylene blue (Terry's modification),[17] and those which were fixed in 10 per cent formalin and stained with hematoxylin and eosin, without embedding.

All specimens were examined with the immersion oil 1.8 mm. objective (N. A. 1.25, tube length 15 mm., and ocular 6×). The shadows of the nuclei and nucleoli were projected, by means of a camera lucida, on paper and traced. The tracing of the nucleoli then had to be enlarged in order to obtain planimeter measurements. For this purpose, we used an objective of 48 mm. (N. A. 0.08, tube length 250 mm., and ocular 1×).

Fig. 2.

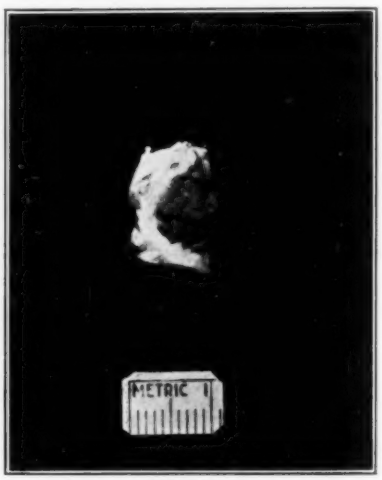

Fig. 3.

Fig. 2.—Single intracystic papillary adenocytoma.
Fig. 3.—Single intracystic papillary adenoblastoma.

Corrections for magnifications were made, and the average size of the nucleus and nucleolus was expressed in square microns. An average of twenty cells was traced from each specimen.

RESULTS OF STUDY

Table I shows the average size of the nucleus and nucleolus, and the nucleonucleolar ratio in four cases of papilloma of the breast.

This table shows that both the nucleus and the nucleolus shrink when the specimen is fixed in a 10 per cent solution of formalin. It also may be noted that the relative shrinkage is greater in the nucleolus than it is in the nucleus. This, in turn, increases the nucleonucleolar ratio.

Table II shows the average size of the nucleus and nucleolus, and the average nucleonucleolar ratio, according to the type of cells, were recorded in thirty-four cases of papilloma of the breast. The specimens in this group of cases

were fixed in a 10 per cent solution of formalin. The average increase in the size of the nucleus and nucleolus is greater in the secondary cytoplastic cell than it is in the reparative regenerative cell, and is greater in the malignant regenerative cell than it is in the secondary cytoplastic cell.

TABLE I

AVERAGE SIZE OF NUCLEUS AND NUCLEOLUS, AND NUCLEONUCLEOLAR RATIO IN FOUR CASES OF PAPILLOMA OF THE BREAST

TYPE OF LESION	NUCLEUS, SIZE IN SQUARE MICRONS		NUCLEOLUS, SIZE IN SQUARE MICRONS		NUCLEONUCLEOLAR RATIO	
	IN FRESH SPECIMENS	IN FIXED SPECIMENS	IN FRESH SPECIMENS	IN FIXED SPECIMENS	IN FRESH SPECIMENS	IN FIXED SPECIMENS
Papillary adenocytoma	34.45	17.80	2.26	0.89	15.78:1	23.30:1
Papillary adenoblastoma	47.08	27.80	3.07	1.68	15.50:1	16.45:1
Papillary adenoblastoma	29.98	20.68	2.08	1.26	15.27:1	17.73:1
Papilloma characterized by secondary cytoplasia*	33.83	29.11	2.09	1.56	17.29:1	19.37:1

*This is a term which MacCarty has used to indicate the occurrence of malignant type of cells which have not migrated to the stroma. Although these cells resemble the cells of cancer, the condition has never been called cancer by MacCarty.

TABLE II

AVERAGE SIZE OF NUCLEUS AND NUCLEOLUS, AND NUCLEONUCLEOLAR RATIO, ACCORDING TO THE TYPE OF CELLS; IN THIRTY-FOUR CASES OF PAPILLOMA OF THE BREAST

CASES	TYPE OF CELL	NUCLEUS, SIZE IN SQUARE MICRONS	NUCLEOLUS, SIZE IN SQUARE MICRONS	NUCLEO-NUCLEOLAR RATIO
12	Reparative regenerative	28.40	1.57	19.55:1
6	Secondary cytoplastic	31.92	2.27	15.08:1
16	Malignant regenerative	33.00	2.62	13.55:1

It is particularly to be noted that the increase in size is relatively greater in the nucleolus than it is in the nucleus. This may be appreciated by glancing at the column in which the ratios are listed. There is a marked absolute and relative increase in the size of the nucleolus of secondary cytoplastic and malignant regenerative cells.

Two hundred and thirty-five specimens were studied in a similar manner, but nuclear and nucleolar tracings were not made.

CONCLUSIONS

1. Of this group of 273 mammary papillomas, 45.6 per cent were papillary adenoblastomas (malignant), while 19.3 per cent revealed secondary cytoplasia, which in all probability is the intermediary stage between benign and malignant conditions. The remaining 35.1 per cent may be classed as papillary adenocytomas (benign).

2. The nucleonucleolar ratio is generally reduced in malignant cells, as compared with cells of secondary cytoplasia and reparative regeneration.

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PULMONARY MONILIASIS*

Report of a Fatal Case

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PART I

REVIEW OF LITERATURE

MONILIASIS is a group of clinicopathologic conditions of the skin, mucous membranes, and internal viscera, associated with the constant presence of monilia fungi in the pathologic lesions, secretions, or excretions of the affected tissues or organs.

The term "Monilia" is a generic one and under it are included a large number of organisms with certain morphologic characteristics which justify their placement in the general class of Hyphomycetes or "*Fungi Imperfecti*" and in the group designated as "yeastlike" fungi. The "yeastlike" fungi include the following genera (Castellani): Cryptococcus, Coccidioides, Saccharomyces, Willia, Torula, Pityrosporum, Endomyces, Monilia, Mycoderma, Oidium, and probably other doubtful genera.

Monilia are widely distributed in nature. In the saprophytic state, they have been found growing on dead leaves, fruits, and decomposed wood. They have also been found in human and animal excreta as well as on the normal mucosa of the mouth, upper respiratory and gastrointestinal tract and skin surface. It is believed that some members of the group which inhabit the human tissue as saprophytes may, under favorable conditions, assume a parasitic rôle, become pathogenic and give rise to cutaneous and visceral disease, and disease of the mucous membranes.

MICROSCOPIC AND CULTURAL CHARACTERISTICS

Monilia reproduce by budding and by the formation of mycelia. The spores vary in size and morphology: large, round, and oval. The mycelia are septate; lateral conidia form by budding near the joints of the hyphae, while terminal conidia form by budding and constriction at the ends of the hyphae. Globular, thick-walled chlamydospores are formed. There are no morphologic variations in the Monilial genus on which one can place a differentiation of the species.

On artificial media, especially those containing glucose, the members of the monilia genus grow rapidly at room or incubator temperature under aerobic conditions. On solid media the growth appears in forty-eight to seventy-two hours as large, round, pasty, moist, white or cream colored

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Received for publication, July 6, 1936.

Read in part before the Clinical Session of the New Jersey Tuberculosis League, December 6, 1935.

colonies resembling colonies of staphylococci. In broth the growth usually consists of a flocculent accumulation of organisms which rapidly settles to the bottom. They grow in gelatin stab cultures along the stab and some strains *(Monilia albicans)* throw out numerous fine lateral branches which become gradually shorter toward the bottom of the stab and give to the growth the "inverted pine tree" appearance originally described by Ashford.

There is considerable controversy as to the reaction of monilia on sugars. Fineman,[1] working with seventeen strains isolated from cases of thrush and supposed to be *Monilia albicans*, finds the fermentation reactions constant. Stovall and Buboiz[2] studied thirty-seven strains of monilia isolated from sputum and found that all organisms showed constant cultural reactions over a period of two years. According to Gay and associates[3] and Jacobson[4] the fermentation of sugars in most species is not constant especially after cultural transplantation. It is the consensus of opinion that *Monilia albicans* is constant in the formation of acid and gas in dextrose, levulose, and maltose.

PATHOGENICITY FOR ANIMALS

Stovall and Pessin[5] were able to classify more than 150 yeastlike organisms associated with various diseases into three species: Type I *(M. parapsilosis)*, Type II *(M. albicans)*, Type III *(M. candida)*. From a series of experiments with rabbits these investigators observed a marked difference in pathogenicity of these three types when injected intravenously. *Monilia parapsilosis* demonstrated no pathogenicity even in doses consisting of three billion organisms. *Monilia albicans*, on the other hand, killed regularly in doses varying from twenty-five to seventy-five million. *Monilia candida* showed a sharp contrast to the other two species. They produced death if very large quantities consisting of six hundred million organisms were injected. Thus, it was found that it required five to fifteen times more cells of *Monilia candida* than of *Monilia albicans* when injected intravenously to kill a rabbit.

Mice may be used also to determine the pathogenicity of monilia. Intraperitoneal injections of *Monilia albicans* give rise to a peritonitis which is usually followed by death in two to ten days. Subcutaneous injections produce localized abscesses at the point of inoculation. When inoculated on the skin of a guinea pig by superficial scarification, *Monilia albicans* produce a mild dermatitis which persists for a week or two during which time the parasites can be found in the scales.

SEROLOGY

Complement fixation reactions, by means of antigens prepared from cultures of the organisms, have been obtained by some investigators with some species of monilia. According to Stone and Garrod[6] the complement fixation and precipitation reactions yield identical results. Agglutination tests are, according to most investigators, unsatisfactory because the organisms show spontaneous agglutination. The precipitin reaction, according to the technic of Stone and Garrod, is of assistance in the determination of species and appears to us to be a more simple and reliable test than the agglutination or complement fixation reaction.

PATHOGENICITY FOR MAN

The relation of monilia to human disease was first discovered by Langenbeck in 1839. He found the fungi in patches of thrush on the oral mucosa, the pharynx, and the whole of the intestinal tract. Herff[7] in 1895 reported twenty-six cases of vaginitis due to monilia. He stated that the organisms produced severe inflammatory reactions which were associated with burning and itching.

Moniliasis of the skin is now accepted as a specific infection. Organisms generally referred to as monilia are regularly found in erosio interdigitalis, in perlèche, in a well-defined type of paronychia, and in diabetic intertrigo.[8] The studies of Benham,[9] Hopkins,[10] Robinson and Moss[11] indicate that almost all of the organisms belong to one well-defined species *(Monilia albicans)*. It is further shown by Benham and Hopkins that in a series of one hundred normal young adults, *Monilia albicans* was not recovered from the skin or the nails in a single case.

It is also said that bone and joint disease may be due to monilia. Case reports can be found by Jacona, Connor, Jones, and Keating.[12]

Kohlbrügge,[13] Ashford,[14] Anderson,[15] and others have described the presence of monilia in the intestines of patients with tropical sprue, but the etiologic relationship of the parasite to this disease is still in doubt. Benham and Hopkins[8] recovered *M. albicans* from the alimentary tract of 18 per cent of the normal persons studied. Ashford[16] reported that 5.6 per cent of 178 healthy boys examined were found to be carriers of monilia as against 52.7 per cent of 74 patients with sprue.

Monilial infection of the bronchopulmonary system was first described by Castellani in Ceylon in 1905. Since then pulmonary moniliasis has been observed, studied, and recorded by a number of investigators in different parts of the world, especially in the tropical countries. Boggs and Pincoffs[17] from Baltimore reported the first case in this country in 1915. Case reports can also be found by Simon;[18] Steinfeld;[19] Johns;[20] Kotkis, Wachowiak, and Fleisher;[21] Wallace and Tanner;[22] Shaw;[23] Stovall and Greeley;[24] Hoffstadt and Lingenfelter;[25] Wheeler and Hoffstadt;[26] Gilbert and Groesbeck;[27] Stokes, Kiser, and Smith;[28] Warr;[29] and Bakst, Hazard, and Foley.[30]

Clinically, Castellani describes three types of bronchopulmonary moniliasis as follows: mild, intermediary, and severe. The mild type affects the patient's general health very little. There is a slight cough, with some mucopurulent expectoration of a yellowish or greenish color, but no blood. Fever is usually absent. In the intermediary type the symptoms are more severe and may simulate early pulmonary tuberculosis. The patient complains of dyspnea and a severe cough. Irregular fever may be present. Hemoptysis may or may not occur. The general clinical picture is that of pulmonary tuberculosis and is characterized by cough, expectoration, hemoptysis, hectic fever, night sweats, and emaciation.

PART II

For seven months we had under our observation a case of chronic pulmonary moniliasis due to *Monilia albicans* which terminated fatally. We are presenting this case along with a report of the constant reactions obtained when this species of monilia is grown in various sugar broths.

REPORT OF A CASE

History.—W. C., white, male, aged forty-five, married, American, a salesman of automobile accessories, was born in Barrie, Vermont, and lived there for twelve years. He worked as a stone carver's apprentice his last year in Vermont. The following eight years were spent in Connecticut. He has lived in Paterson, New Jersey, for the past twenty-five years. The patient has never traveled in the midwest or in the tropics.

For several months before admission to the hospital he felt increasing languor and fatigue. There was no loss of weight. About May 1, 1935, he developed a severe pain in his lower left chest and upper left abdomen which was more severe on deep inspiration. At this time there was no cough or fever. Four weeks later he developed a nonproductive cough which

Fig. 1.—Roentgenogram made June 26, 1935. *Right Lung*: There is a dense shadow over the apex. The lung details are not clearly visible. There is some slight mottling in the first and second interspaces. No definite area of cavitation can be accurately outlined. *Left Lung:* There is rather dense shadow over the apex. There is some infiltration immediately below the first rib. Some ringlike shadows in the lower lobe which possibly represent dilated bronchi.

was followed in a few days by expectoration of about five or six ounces of purulent sputum daily. The quantity of sputum gradually increased to twelve ounces daily. The temperature varied from 100 to 103° F. The condition failed to improve and he was admitted to Valley View Sanatorium on June 26, 1935 (about two months after the development of chest pains and cough), with the chief complaint of productive cough (16 ounces daily), dyspnea, bilateral pleurisy, anorexia, profuse night sweats, constipation, pain in the right abdomen, and a loss of about 10 pounds in weight.

Physical examination revealed a well-developed, slightly emaciated, very dyspneic and weak individual with a temperature of 101° F. Mucous membranes of the mouth and throat were negative. There was no lymphadenopathy. There was pain on pressure along the left

costal margin and right abdomen. The liver was not palpable and no masses could be felt in the abdomen. The percussion note was impaired over the right lung and a few fine râles were heard in the upper lobe. There was distant breathing over the upper lobe of the left lung. The roentgenogram on admission showed infiltration of the first and second interspaces on the right, and an area in the apex and first interspace on the left which was interpreted as probably cavitation (Fig. 1).

Clinical Course (Table I).—During the first two weeks of residence his condition remained essentially unchanged, except for an acute abdominal condition which was probably a subacute attack of appendicitis. After two weeks he began to gain strength, night sweats disappeared, cough was markedly decreased. Expectoration decreased rapidly. The disappearance of dyspnea was the most striking change. The sputum always remained thick, yellow with very slight amount of saliva. At the end of two and a half months, the patient's sputum was reduced to one ounce daily. He gained several pounds in weight, and felt so well that he became impatient because he was kept in bed.

Fig. 2.—Roentgenogram made Dec. 6, 1935. *Right Lung*: Several large areas of consolidation throughout the upper three-fourths of the lung. Few areas of small honeycombed cavitation in the first and second interspaces. *Left Lung*: Few areas of consolidation with intervening areas of honey-combed cavitation in the first and second interspaces.

The symptoms reappeared very suddenly after one month. They were progressively worse during the remainder of his life. Two weeks following the relapse, he developed a severe sore throat which was refractory to treatment. It was accompanied by hoarseness and dysphagia. An examination revealed a generalized redness, but no ulceration.

One month following the relapse the patient complained of extremely severe dyspnea and weakness. The sputum was blood streaked and was markedly reduced in quantity. Dur-

ing the next four weeks, he had practically no expectoration. Fever became subnormal and never returned to normal. Three weeks later he was stricken with the sudden onset of extremely severe abdominal pain, the greatest intensity was in the umbilical region.

Marked tenderness was revealed on palpation. The next day the pain became cramplike, followed the course of the transverse and ascending colon, was wavelike and was synchronous

TABLE I

RÉSUMÉ OF CLINICAL COURSE AND TREATMENT

DATE	REMARKS
7/10/35	Two weeks after admission, night sweats, cough, and expectoration decreased. Temperature unchanged.
7/15/35	Potassium iodide gtts. v. t. i. d., increased as tolerated to gtts. xxx t. i. d. Reaction Aug. 20, 1935, dosage reduced, given as tolerated until Nov. 25, 1935.
7/24/35	Night sweats disappeared. Cough and expectoration decreased to 10 ounces daily. Strength increased. X-ray reveals decrease in infiltration of upper lobes bilaterally. Temperature 98°-100° F. Pulse 86-120.
9/ 5/35	Expectoration decreased to 1 ounce daily. General condition improved. Temperature 98°-99.2° F. Pulse 80-110. X-ray: Right Lung: No change. Left Lung: Possibly a small cavity in second interspace.
9/27/35	Intradermal injection of 0.1 c.c. Monilia vaccine (prepared by Drs. Smith and Martin of Duke University). Slight reaction in forty-eight hours. General condition improved. Temperature and pulse unchanged. Auscultation reveals scattered moderately coarse râles in both lungs.
10/15/35	0.1 c.c. of monilial vaccine, subcutaneously. Vaccine was given twice a week for five weeks, increasing the dose 0.1 c.c. each injection.
11/ 5/35	Severe cough. Expectoration of ounces viii daily. Temperature 99-101° F. Pulse 96-130. X-ray: Right Lung: Apparently a consolidation in apex. Left Lung: Increase in infiltration first and second interspaces.
11/26/35	X-ray: Left Lung: Increase in infiltration in apex and hilus. Right Lung: Increase in infiltration throughout. Areas of small multilocular cavities first and second interspaces. Dilatation of heart. Auscultation, evidence of pulmonary edema. Soft blowing murmur at apex.
11/30/35	Severe sore throat, hoarseness, dysphonia, and dysphagia for one week. Inspection reveals redness of mucous membrane of throat but no ulceration. General condition is worse. Sputum blood streaked.
12/ 6/35	X-ray: Right Lung: Further extension of consolidation toward base. Left Lung: Further extension of consolidation in the upper lobe (Fig. 2).
12/ 7/35	Slight generalized edema. Temperature 96-98° F. Pulse 96-120.
1/28/36	Steady loss of strength. Practically no cough and expectoration. Acute generalized pain of abdomen, most severe at umbilicus. Abdomen rigid. No masses felt. Pain, cramplike in character. After two or three days the tenderness was most severe in region of appendix. Tenderness persisted until death. Temperature subnormal. Pulse 80-100.
2/ 7/36	Vomiting large quantities of coffee ground material. The stool is black. Died. Autopsy refused.

TABLE II

ROUTINE BLOOD COUNTS AND HEMOGLOBIN

	6/7/35	7/24/35	8/3/35	9/20/35	11/8/35	11/22/35
Hb.	75%	50%	55%	70%	45%	45%
R.B.C.	3,870,000	2,960,000	2,980,000	3,960,000		2,980,000
W.B.C.	13,900	20,000	10,200	9,200	14,600	16,200
Neut.	73%	74%	80%	73%	80%	73%
Eosin.	1%	1%	1%	1%	1%	1%
Baso.	1%	1%	1%	1%	1%	1%
Lympho.	20%	18%	13%	19%	14%	20%
Mono.	5%	6%	5%	6%	4%	5%

with peristalsis. There were no masses. After two days the pain disappeared, but the entire abdomen was tender upon pressure. The liver became palpable with the lower edge about four inches below the costal margin. Two days prior to death patient began to vomit large quantities of coffee ground vomitus. Patient died Feb. 7, 1936.

Blood Picture.—The blood picture was that of a low-grade infection and progressive secondary anemia (see Table II). The highest number of leucocytes recorded at any time was 20,000 per c.mm. with an average of 14,000, and the highest percentage of polymorphonuclear leucocytes was 80 per cent, with an average of 76 per cent. Eosinophiles were not increased.

Routine Laboratory Findings.—Twenty-seven specimens of sputums collected at various times during the period of hospitalization were found by the direct smear to be negative for tubercle bacilli. On many occasions the report was positive for fungi. The stool on three occasions and the urine on two occasions were found positive for fungi which were later identified as *Monilia albicans.* The urine also showed the constant presence of albumin and many epithelial and pus cells. The blood chemistry, blood culture, Kahn and the agglutination test for undulant fever, typhoid, paratyphoid "A" and "B" fever were negative.

We made a special study of the yeastlike organisms that were routinely found in the sputum and similar organisms that we also isolated from the sputum on several occasions and identified them as *Monilia albicans.* Pure cultures were obtained by streaking fresh sputum on Sabouraud's agar plates and slants and incubating at room temperature. The suspicious

Fig. 3.—A seventy-two-hour broth culture of *Monilia albicans* photographed from a "broth agar hanging drop" preparation.

colonies were picked and planted in nutrient broth, incubated at 37° C. for twenty-four hours and examined microscopically for the presence of extraneous organisms. On Sabouraud's dextrose agar the colonies were large, white, moist, and pasty. After three to five days the organisms could be seen growing down into the mediums in a fringe fashion.

We find that a very convenient preparation for the microscopic study of the unstained organisms as they are found growing in liquid media is the "broth agar hanging drop"* preparation (Fig. 3). The organism produced definite mycelia, made up of elongated cells with definite septate hyphae. Spores varied in size and shape. They were large, small, round, or oval. Lateral conidia formed by budding near the joints of the hyphae, and terminal conidia by budding and constriction at the ends of the hyphae. Globular thick-walled chlamydospores were formed.

*The "broth agar hanging drop" is prepared by adding one loopful of melted agar (cooled to 45° C.) to a loopful of broth culture of the organisms on an ordinary hanging drop slide. The agar congeals and forms a stable preparation, which prevents Brownian movement of the organisms, and will keep for three or more weeks provided vaseline is applied around the margin of the cover glass to inhibit drying.

CULTURAL CHARACTERISTICS

Nutrient Broth.—There appeared in twenty-four to seventy-two hours a flocculent, white, fluffy growth which gradually settled to the bottom of the tube.

Sugar Broth.—Acid and gas were formed in mannose, maltose, dextrose, and levulose. Acid without the production of gas was formed in dextrine, galactose and saccharose. There was no production of gas or acid in arabinose, mannite, lactose, xylose, dulcite, and rhamnose during ten days incubation at 37° C.

Litmus Milk.—There was no change during five weeks incubation at 37° C.

Gelatin.—Gelatin stab—There appeared an "inverted pine tree" growth in seventy-two hours at room temperature. There was no liquefaction during five weeks incubation at 37° C.

Endo's Medium.—After six days' incubation at room temperature the colonies appeared as elevated white growths. The mycelia could be seen radiating from the center-like spokes from the hub of a wheel. The mycelia penetrated into the medium, thus making it impossible to remove the entire colony without distorting the medium.

Since it is claimed by most investigators that monilia is not constant in its reaction on various sugars, we subjected this strain of *Monilia albicans* to different environments and then determined the reaction on 1 per cent sugar broths (Table III). Group A represents the organisms isolated from the sputum. Group B represents the organisms isolated from the kidney of a rabbit which had been injected with organisms of Group A. Group C represents organisms from Group A which had been transplanted to various media including sugar broths for six transplants. Group D represents organisms isolated from the feces of a white mouse three days after the feeding of the

TABLE III

REACTION OF *Monilia albicans* ON 1 PER CENT SUGAR BROTHS DURING TEN DAYS' INCUBATION AT 37° C.

GROUPS	A	B	C	D	E	F
Mannose	AG*	AG	AG	AG	AG	AG
Maltose	AG	AG	AG	AG	AG	AG
Dextrin (X)	A	A	A	A	A	A
Lactose	O	O	O	O	O	O
Dextrose	AG	AG	AG	AG	AG	AG
Galactose	A	A	A	A	A	A
Mannite	O	O	O	O	O	O
Saccharose	A	A	A	A	A	A
Levulose	AG	AG	AG	AG	AG	AG
Dulcite	O	O	O	O	O	O
Arabinose	O	O	O	O	O	O
Rhamnose	O	O	O	O	O	O
Xylose	O	O	O	O	O	O

*AG, Acid and Gas. A, Acid only. O, No reaction. X, From corn.

organisms of Group A to the mouse had been discontinued. Group E same as Group D except the organisms were isolated twelve days later. Group F same as Group D except that the organisms were isolated twenty days after the feeding of the organisms was discontinued. It was observed that the

reaction of the various sugar media was constant. In some cases the final reaction was obtained in twenty-four hours, whereas in others it required several days.

ANIMAL INOCULATIONS

A young female rabbit, weight 4.5 pounds, was injected intravenously with 1 c.c. of a saline emulsion consisting of 42 million organisms from a forty-eight-hour growth on Sabouraud's agar. At the end of seventeen days the animal died. At autopsy the kidneys were swollen about twice their normal size. Grayish white abscesses were found throughout the cortex and medulla, but most pronounced in the former. An abscess about 1 cm. in diameter was located in the left lobe of the cerebellum. Other organs were apparently normal. The monilia were isolated in pure culture from the kidneys.

Two white mice were inoculated subcutaneously with 210 million and 420 million, respectively, of the organisms isolated from the sputum. The mice did not appear to be ill. They were killed fourteen days after inoculation. Each mouse showed a well-defined abscess about 2 cm. in diameter located at the point of inoculation. The abscess consisted of grayish white, caseous material. Monilia were isolated from the abscess of each mouse. All internal organs were normal.

A young female mouse, weight 9 gm. was injected intraperitoneally with 41 million of the organisms which were isolated from the sputum. The mouse died in eight days. Autopsy showed extensive grayish white necrosis of the omentum with similar abscesses in the liver, spleen, and kidneys. The organisms were recovered from the kidneys and spleen. A white adult mouse was injected intraperitoneally with 42 million cells. The mouse appeared inactive and drowsy after five days. Eight days after injection, it was killed. At autopsy a grayish white necrotic area was found in the omentum from which the monilia were isolated.

Four guinea pigs were injected intraperitoneally with sputum during the patient's illness. On July 3, 1935, a pig was inoculated with about 30 c.c. of sputum concentrated by the oxalic acid method. This was repeated on a second pig Dec. 4, 1935. The third one was inoculated with 1 c.c. of non-concentrated sputum on July 17, 1935 as was the fourth on Nov. 8, 1935. The four pigs were killed and autopsied eight weeks after inoculation. There was no evidence grossly or microscopically of tuberculosis or fungus infection. Cultures on Sabouraud's medium from the peritoneum and spleen failed to reveal any monilia or other organisms.

SEROLOGIC EXAMINATION

A vaccine consisting of 10 c.c. of a heavy emulsion in saline was prepared from a pure culture of *Monilia albicans* which was received from Miss Rhoda W. Benham of Columbia University. Three injections consisting of 3 c.c., 3 c.c., and 4 c.c. during a period of three weeks were given intravenously in the ear vein of a young adult rabbit. Two months after the first injection 5 c.c. of blood were obtained from the ear vein. The titer of the precipitins was found to be 1:400 when an extract of the known *Monilia albicans* was used. A precipitation in dilution as high as 1:400 was also found with an extract prepared from the organisms isolated from the sputum of this case.

The blood serum obtained from the patient September 25, and October 21, showed no agglutination in dilutions of 1:10, 1:20, 1:80, and 1:160 of the monilia isolated from the sputum.

PART III

SUMMARY

We have presented herein a report of a fatal case of pulmonary moniliasis due to *Monilia albicans* including a review of the literature on the same subject. The morphologic, cultural, and serologic characteristics and the pathogenicity of monilia for man and laboratory animals have been discussed. The strain of *Monilia albicans* isolated from the sputum of this patient was subjected to different environments and the reactions on nutrient broths, each containing 1 per cent mannose, maltose, dextrin, lactose, dextrose, galactose, mannite, saccharose, levulose, dulcite, rhamnose, arabinose, and xylose were determined. Our findings definitely prove that the reaction of this strain of monilia was constant on all the sugars.

The monilia in the case reported was found to be pathogenic for rabbit when injected intravenously; for white mice when injected subcutaneously or intraperitoneally; but was not pathogenic for guinea pigs when 1 c.c. of sputum containing the organisms was injected intraperitoneally.

A simple and useful preparation for the study of broth cultures of unstained monilia is described.

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CREATINURIA IN SCLERODERMA*

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THE skeletal muscles of patients with scleroderma may show either atrophy due to disuse or actual sclerotic changes occurring in their substance. The realization that these processes were occurring suggested the investigation of the amount of creatine excreted in the urine of patients suffering with this disease. The following six patients, the only ones tested, showed a creatinuria, the significance of which is not clear at the present time.

REPORT OF CASES

Case 1.—Mrs. L. B., a sixty-three-year-old negress, presented a symmetrical scleroderma of ten years' duration involving both lower legs. The process extended from just below the knees to a point distal to the ankles. The upper three-fourths were in an atrophic stage and showed complete depigmentation. The lower one-fourth of the leg was shiny, hyperpigmented, and indurated. The diagnosis was confirmed by histologic studies. The basal metabolic rate was plus 6 per cent.

The urinary creatine and creatinine were studied with the following results:

	Nov. 4	Nov. 18	Dec. 2	Dec. 9
Creatine	0.351†	0.368	0.500	0.198
Creatinine	1.253	0.517	1.140	0.565
Total	1.604	0.885	1.640	0.763

The patient was treated by the oral administration of 40 gm. of aminoacetic acid three times a day. Although this therapy was continued from November 18, 1935, to February 10, 1936, a total of almost three months, no clinical improvement was noted.

Case 2.—Mrs. M. K., Japanese, aged twenty-six years, presented a generalized scleroderma of one year's duration. Biopsy revealed changes characteristic of scleroderma. Sclerodactylia was marked. Her basal metabolic rate was plus 12 per cent. Studies performed on her urine showed:

Creatine	0.275 gm. excreted in the urine in 24 hours
Creatinine	0.543 gm. excreted in the urine in 24 hours
Total	0.818 gm. excreted in the urine in 24 hours

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Received for publication, August 20, 1936.
†Values expressed in grams excreted in urine in twenty-four hours.

She was given one and one-half ounces of aminoacetic acid daily for two weeks, and no change could be seen in her condition.

CASE 3.—Mrs. B. S., aged thirty-six years, presented a scleroderma of eight years' duration that involved the face, neck, and hands. Her basal metabolic rate was plus 8 per cent. Biopsy confirmed the diagnosis. Analysis of her urine gave the following results expressed in grams excreted in twenty-four hours:

	Oct. 14	Oct. 21	Oct. 22
Creatine	0.430	0.720	0.400
Creatinine	1.040	1.330	1.140
Total	1.470	2.050	1.540

Aminoacetic acid therapy was attempted in this patient but was discontinued because of nausea and vomiting.

CASE 4.—Mrs. D. P., aged fifty-two years, presented a generalized scleroderma of two years' duration. The biopsy confirmed the clinical diagnosis. Her basal metabolic rate was minus 7 per cent. Urinalysis showed:

Creatine	0.884 gm. excreted in the urine in 24 hours
Creatinine	0.824 gm. excreted in the urine in 24 hours
Total	1.708 gm. excreted in the urine in 24 hours

This patient did not receive any aminoacetic acid.

CASE 5.—Miss R. B., a negress, aged twenty-six years, had suffered from scleroderma of the face, neck, and hands for six years. Sclerodactylia was very marked. Her basal metabolic rate was minus 5 per cent. Histopathologic studies confirmed the diagnosis.

A twenty-four-hour urine specimen showed:

Creatine	0.253 gm.
Creatinine	0.775 gm.
Total	1.028 gm.

Aminoacetic acid therapy was not given a trial in this patient.

CASE 6.—Mrs. B. H., aged sixty-four years, had had scleroderma of the face, neck, hands, and feet for a period of six years. Her basal metabolic rate was minus 4 per cent. Biopsy revealed changes in the skin characteristic of the stage of induration of scleroderma.

Urinalysis showed:

Creatine	0.086 gm. excreted in 24 hours
Creatinine	0.438 gm. excreted in 24 hours
Total	0.524 gm. excreted in 24 hours

She received 40 gm. of aminoacetic acid and 0.0225 gm. of ephedrine sulphate orally twice a day for a period of twelve weeks. No subjective or objective improvement was noted.

DISCUSSION

Creatinuria normally occurs in children and in women during the menstrual period and during pregnancy.[1] It is believed that this can be explained on the basis of deficient muscular development in such persons in comparison with that found in the adult male, or in differences in the sex glandular apparatus.

The quantity of so-called "normal creatinuria" in women is of extreme importance in interpreting the results of these studies. Mathews[2] has shown that the normal creatinuria is most marked in girls at the age of seven years and slowly decreases thereafter. According to Macleod,[1] the amount of creatine

excreted in the urine during pregnancy is seldom greater than 0.1 gm. per 24 hours. Hodgson and Lewis[3] reviewed the subject in 1928 and found reports of 222 urine specimens collected from normal women that had been tested for creatine content. Of these, only 103 (46.3 per cent) revealed creatinuria. The values ranged from 0.04 gm. to 0.32 gm. excreted in 24 hours. The above authors tested the urine of 14 female patients, 2 specimens from each being analyzed. Twenty-seven of the 28 specimens contained some creatine, the amounts varying from 0.02 to 0.17 gm. in a 24-hour sample. However, 23 of these presented values lower than 0.100 gm., and only 1 patient had 2 specimens with a creatine content in excess of this figure.

In comparison, our patients showed a creatinuria ranging from 0.086 to 0.884 gm. per day. All 11 specimens were positive and 7 of them were greater than 0.32 gm., the highest figure quoted above. Of the 4 below this figure, one of the patients had three other urinalyses showing a creatine content of more than 0.35 gm. Two of the others falling in this category gave values of more than 0.250 gm. of creatine excreted in the urine in 24 hours.

In no instance was the patient either pregnant or menstruating when the tests were performed; in fact, 3 of them had passed through the menopause. All were on a comparatively high carbohydrate, low protein diet when the first tests were done. The urinary creatinine was within normal limits in each of the 6 cases reported.

Other conditions that may cause creatinuria include acidosis (starvation, diabetes mellitus, fever), severe liver damage (infectious, neoplastic, or toxic), muscular atrophies and dystrophies, myasthenia gravis, hyperthyroidism, and wasting diseases. The findings in scleroderma are probably to be explained on the basis of muscular atrophy.

The results obtained by the administration of aminoacetic acid (glycine) were disappointing. This can be explained by conceding that the creatinuria is a secondary effect of scleroderma, a disease probably having a different etiology from that of myasthenia gravis and the muscular atrophies and dystrophies that usually respond to such therapeutic measures. Furthermore, in scleroderma, the pathologic alterations are not confined to the muscles but also involve the skin and other portions of the body.

SUMMARY

1. The occurrence of creatinuria in six patients with scleroderma is reported.

2. An attempt is made to explain the failure of aminoacetic acid therapy in these cases.

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VISIBLE ACTION OF SODIUM LAURYL SULPHATE ON MICROORGANISMS*

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SODIUM lauryl sulphate is the sodium salt of the sulphate ester of lauryl alcohol and has the following chemical structure:†

$$CH_3(CH_2)_{10}COSO_3Na$$

Because of its surface tension depressing properties, combined with its solubility in the presence of calcium and magnesium ions and in the presence of acid or alkali, it was thought that this substance should prove useful in bacteriology where such substances as sodium oleate, sodium ricinoleate, bile and bile salts are used. Sodium lauryl sulphate has been shown to have germicidal properties similar to such soaps as sodium oleate and sodium ricinoleate and has also been shown to have the ability to neutralize diphtheria toxin in vitro (Bayliss and Halvorson, 1935).

Sodium ricinoleate, which has properties similar to this compound, has been studied in its visible action on microorganisms by various workers. Kozlowski (1928) has reported that sodium ricinoleate, under the proper conditions, clears suspensions of pneumococci with an increase in viscosity. Streptococci under the same conditions remain unchanged, so that this substance can be used in place of bile for the bile solubility test for pneumococci. Spencer (1930) has found that a large number of microorganisms are visibly affected by treatment with varying amounts of sodium ricinoleate. Certain species of bacteria, after washing and suspending in physiologic saline, were rendered water clear while other species increased in turbidity. Some species increased in turbidity at one concentration and decreased at another concentration. *Pasteurella tularensis* was completely cleared in low concentrations of castor oil soap. He found pneumococci to be only slightly altered.

EXPERIMENTAL

The action of sodium lauryl sulphate was studied by adding from 0.1 to 1.0 c.c. of various concentrations of crude sodium lauryl sulphate to twenty-four-hour peptone cultures of about 4 c.c. volume. The peptone medium con-

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Received for publication, August 25, 1936.

†"Dreft," which is an impure form of this same compound manufactured by Procter and Gamble Company, contains about 50 per cent sodium sulphate. Of the organic substances, about 25 per cent of the total is sodium lauryl sulphate. The other 25 per cent consists of compounds very similar both in structure and properties to this compound, such as sodium sulphate esters of decyl, myristyl, cetyl and oleyl alcohols. The normal variation of composition of the organic materials corresponds to that of coconut oil and palm kernel oil from natural sources. "Dreft" as it is marketed gives a pH of approximately 7 when dissolved in concentrations of 2 per cent and less as measured by the glass and hydrogen electrodes.

"Dreft" is soluble in water up to around 20 per cent at 25°C. At lower temperatures a portion of the material will precipitate at this concentration. A 20 per cent solution is slightly yellow after a light flocculent precipitate, apparently ferric hydroxide, is allowed to settle out or is centrifuged. Solutions of 5 per cent and less are clear and colorless after the minute amount of precipitate is allowed to settle.

tained 1 per cent peptone and 0.5 per cent sodium chloride. In the case of those organisms for which the peptone proved unsuitable for growth, such as the pneumococci, streptococci, and the anaerobic organisms, deep tubes of brain broth were used and the upper layers of the fluid removed following growth for the clearing test. Organisms grown on agar slants and suspended in saline or peptone solution did not always give the same results as obtained from growth in liquid media. If the culture was not sufficiently turbid in twenty-four hours, it was allowed to grow for a longer period of time until the turbidity was equal to, or greater than, No. 1 standard on the McFarland turbidity scale (1907). Controls of the uninoculated media plus sodium lauryl sulphate and cultures without this compound were always made for comparison. In most cases, however, this is unnecessary as the results are quite striking. The increase in viscosity was determined by shaking the tubes vigorously and observing the ascension of the air bubbles through the liquid.

DISCUSSION

Sodium lauryl sulphate has the ability to clear suspensions of gram-negative organisms and pneumococci and at the same time produce a marked increase in viscosity when these organisms are grown in a liquid medium such as peptone or veal broth. The gram-negative organisms show marked evidence of clearing after fifteen minutes' incubation at 37° C. By the end of one hour (Table III) the reaction has apparently almost reached completion as only slight changes are noted after four and twenty-four hours. The organisms were also observed at the end of forty-eight hours but only in exceptional cases was any difference noted from the twenty-four-hour reading. Although there is a marked decrease in the turbidity of the suspension with an increase in the viscosity of the medium, very little difference is noted microscopically. Viewed in hanging drop preparations no marked change is noted in the bacterial cells. Under dark-field illumination and with stained preparations, including negative stains, the organisms appear to retain their original size and form. If there is any change it would be for the organisms to become slightly smaller, but this also seemed to be true of the gram-positive organisms which were unaffected macroscopically. However, microscopic observations were made on only a few of the organisms.

There was no correlation between the clearing action and the lethal action of the sodium lauryl sulphate. Some of the organisms which were cleared were killed while others were not. The same was true of those which were not visibly affected.

The gram-negative organisms fall roughly into two groups (Table I). The vibrios are cleared in dilutions of 1/2,560 or greater while the remaining organisms are cleared in dilutions of 1/320 or less during the one-hour period.

Gram-positive organisms in general are completely unaffected either in turbidity or in the viscosity of the culture (Table II). In a few cases, notably *Bacillus megatherium* and *Torula cremoris* when twenty-four-hour peptone cultures were used, the turbidity was slight, and this turbidity would decrease upon treatment with the surface tension depressant without any notice-

able increase in viscosity. If, however, the culture were allowed to grow for a week, these organisms then appeared to be unaffected. Seven-day cultures of *Escherichia coli* were cleared in the same manner as twenty-four-hour cultures.

TABLE I

GRAM-NEGATIVES. HIGHEST DILUTION OF CRUDE SODIUM LAURYL SULPHATE SHOWING GREATER THAN 50 PER CENT CLEARING IN ONE HOUR

Aerobacter aerogenes	1/160	*Pseudomonas aeruginosa*	1/320
Aerobacter cloacae	1/320	*Salmonella enteritidis*	1/160
Alcaligenes faecalis	1/160	*Salmonella paratyphi*	1/80
Brucella abortus	1/40	*Salmonella schottmülleri*	1/160
Eberthella typhi	1/320	*Salmonella pullorum*	1/320
Escherichia coli communis	1/160	*Salmonella typhi murium*	1/320
Escherichia coli communior	1/160	*Serratia marcescens*	1/320
Klebsiella capsulatus	1/160	*Shigella dysenteriae*	1/320
Klebsiella pneumoniae	1/160	*Shigella paradysenteriae*	1/160
Neisseria catarrhalis	1/40	*Vibrio comma*	1/2,560
Neisseria meningitidis	1/320	*Vibrio metchnikovi*	1/2,560
Proteus vulgaris	1/320	*Vibrio priori*	1/5,120
Proteus X19	1/320		

TABLE II

GRAM-POSITIVES—NOT CLEARED

Actinomyces gypsoides	*Micrococcus flavus*
Bacillus anthracis	*Monilia albicans*
Bacillus cereus	*Mycobacterium tuberculosis*
Bacillus mesentericus	*Rhizopus nigricans*
Bacillus mycoides	*Sarcina lutea*
Bacillus subtilis	*Saccharomyces cerevisiae*
Bacillus terminalis	*Saccharomyces lemmonieri*
Clostridium sporogenes	*Saccharomyces* (sp.)
Corynebacterium diphtheriae mitis	*Staphylococcus aureus*
Corynebacterium diphtheriae gravis	*Staphylococcus citreus*
Corynebacterium diphtheriae intermediate	*Staphylococcus roseus*
Corynebacterium hoffmani	*Streptococcus* (hemolytic)
Corynebacterium xerosis	*Streptococcus* (nonhemolytic)
Diphtheroid (bright orange)	*Streptococcus lactis*
Micrococcus cereus	*Streptococcus (viridans)*
Geotrichum lactis	*Torulopsis mucilaginosa*

TABLE III

DIPLOCOCCUS PNEUMONIAE (TYPE I)

	15 MIN.	1 HR.	4 HR.	24 HR.
1/20	4*	4	4	4
1/40	4	4	4	4
1/80	4	4	4	4
1/160	4	4	4	4
1/320	4	4	4	4
1/640	4	4	4	4
1/1,280	4	4	4	4
1/2,560	4	4	4	4
1/5,120	2	4	4	4
1/10,240	–	2	3	3
1/20,480	–	–	1	2
1/40,960	–	–	–	1
1/81,920	–	–	–	–
0	–	–	–	–

*4, 90-100 per cent clearing with marked increase in viscosity.
3, 50-90 per cent clearing with increase in viscosity.
2, 25-50 per cent clearing.
1, 10-25 per cent clearing.
–, Less than 10 per cent clearing.

Diplococcus pneumoniae falls in a class by itself. It is the only gram-positive organism in this series which was rapidly cleared in high dilutions (Table III). From its ready solubility in bile and bile salts one would expect it to be soluble in a substance with somewhat similar properties, such as sodium lauryl sulphate. Because of the ease of clearing of these organisms all strains of pneumococci available have been tested with this substance in a concentration of 1/500. The test was performed by adding to a culture of the organism one-tenth of its volume of a 2 per cent solution of sodium lauryl sulphate. The culture was then observed after thirty minutes at 37° C. All cultures of pneumococci, including the four main groups, were cleared with an increase in viscosity when the growth was profuse. All streptococci tested under such conditions were unaffected. The ease of obtaining satisfactory material for carrying out this test and of reading the results, because of the water clearness of the solution of sodium lauryl sulphate, seem to make this procedure a desirable substitute for the bile solubility test. However, the number of strains of pneumococci and streptococci tested in this series has been limited.

The only gram-positive organisms in this series other than the pneumococci showing definite clearing with a marked increase in viscosity was *Bacillus laterosporus.*

Churchman (1928) has listed the principle differences between the gram-positive and the gram-negative organisms. According to various workers the latter group is more readily subject to autolysis, serolysis, and dissolution by 1 per cent sodium hydroxide. These statements point to the fact that the nature of the membrane surrounding the protoplasm is of a different nature in the case of the gram-negative bacteria and more readily subject to alteration. In view of the fact that the organisms appear in the main to be unchanged microscopically by this compound, it would appear that the clearing is due mainly to a change in the refractivity of the bacterial cell. However, the marked increase in viscosity at the same time would indicate that either a portion of the bacterial cells are being disintegrated or that certain units of the bacterial protoplasm are being dispersed throughout the medium.

Heating alters the gram-negative organisms so that they are no longer cleared by the action of this surface tension depressant. Neither *Escherichia coli* nor *Staphylococcus aureus* which have been autoclaved are altered in any manner by this compound. It clears gram-negatives which have been treated with dilute ethyl alcohol, acetone or ether, but not if they have been treated with formaldehyde. The addition of dilute acid to the culture prevents the clearing action of the sodium lauryl sulphate, whereas the addition of alkali hastens the clearing of gram-negative bacteria.

SUMMARY

Cultures of gram-negative organisms grown in liquid media are cleared by appropriate concentrations of sodium lauryl sulphate. At the same time there is a marked increase in the viscosity of the medium.

Vibrios are cleared in dilutions up to 1/2,560 or greater in one hour while other gram-negative bacteria are cleared in dilutions only up to 1/320 or less.

Pneumococci are also cleared in dilutions up to 1/5,120 in a one-hour period. Streptococci are unaffected. Most other gram-positive bacteria remain unchanged.

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THE INFLUENCE OF SODIUM AMYTAL UPON THE BLOOD AND URINE UREA NITROGEN*

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THERE are very little data in the literature concerning the effect of sodium amytal, given in large doses, on the ability of the kidney to excrete urea. Since the volume of urine is decreased after sodium amytal is given, it is often implied that kidney function is reduced. Therefore, we are reporting results on an extensive series of dogs, so that more data may be available for those who contemplate studies of kidney function on anesthetized dogs.

There is no doubt that under the influence of sodium amytal the rate of secretion of the urine is diminished. (Bourne, Bruger and Dreyer,[1, 2] Ogden,[3] Emge and Hoffman,[4] Walton.[5]) There is also dilution of the blood. (Bourne, Bruger and Dreyer[1, 2] and Adolph and Gerbasi.[6])

The quantity of urea secreted per minute was found to be diminished by Bourne and coworkers.[1] Karr and Nasset[7] reported an average value of 14.9 mg. per cent for blood urea before anesthetization with sodium amytal and 14.8 after anesthesia (2¼-5½ hr.).

METHODS

The 50 female dogs used in these experiments ranged in weight from 4.5 to 13.2 kg. They were fasted for twenty-four hours before being used for experiment. The first blood sample from either the saphenous or jugular vein was taken in each case immediately before administration of the anesthetic. Sodium amytal was then given intraperitoneally, 60 mg. per kg. It was sometimes necessary to administer smaller doses (50 to 100 mg. per dog) at various times throughout the day in order to obtain an even degree of deep anesthesia. Two additional blood samples were collected at approximately one and one-half and three hours, respectively, after giving the sodium amytal.

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Received for publication, August 21, 1936.

As soon as the dog was well under the influence of the anesthetic, a catheter was inserted into the bladder and thorough washing carried out. The bladder was again washed two and one-half to three hours later and the sample obtained analyzed for urea nitrogen. Since all authors agree that the quantity of urine secreted is diminished after sodium amytal, no attempt was made to measure the volume. Values are expressed in milligrams per hour. The only operative procedure carried out during this time was the insertion of a tracheal cannula.

Both blood and urine nitrogen determinations were made by the urease aeration method of Meyers.[8] Urease was prepared by the method of Koch.[9]

RESULTS

The results are presented in Tables I and II. The values for blood urea nitrogen (Table I) before anesthetization range from 3.4 to 34.4 mg. per cent with an average of 11.7. These values are in accord with those of Haden and Orr[10] who reported in a series of 200 determinations on normal dogs a range from 5.1 to 42.1 mg. per cent with an average value of 11.7. The average for the samples taken approximately one and one-half hours after administration of the anesthetic is 11.9, indicating no significant change. There is, however, a tendency toward a decrease in those animals having a high initial level as noted with Dogs 8, 10, and 43, but this does not always occur, as seen in Dogs 11 and 17. Dogs starting at lower levels tend to remain very nearly the same or to fluctuate somewhat in either direction. In most cases the same tendencies seem to be continued for the three-hour period where the average value is 12.0 mg. per cent.

Urine urea nitrogen values expressed as milligrams per hour show wide variations ranging from 29.8 to 411.6. These values appear somewhat lower than those given in the literature for normal dogs but are very likely explained by the small size of the dogs and their failure to consume large quantities of meat, particularly during the warmer weather when much of the work was in progress.

In Table II we have given values for blood urea nitrogen for five consecutive hours after the intravenous administration of 10 gm. of glycine. Unfortunately in the cases of Dogs 3 and 4, blood samples were not taken before sodium amytal was given, but the blood urea nitrogen of Dog 3 was approximately back to its initial value five hours after the administration of the glycine. This dog had excreted 90 mg. of urea nitrogen per hour before the glycine was administered, and during the five hours afterward he excreted 1,669 mg. or an excess over the basal excretion of 1,088 mg. The blood urea nitrogen of Dog 4 fell well below the initial level at the end of the five-hour period. Likewise Dogs 28 and 31 had lower blood urea nitrogen figures at the end of the experiment than at the beginning. They excreted a total of 573 and 260 mg., respectively, of urine urea nitrogen above the basal level. Dog 31 clearly did not metabolize so much glycine.

Dogs 29 and 32 were a little above the initial blood urea nitrogen level at the end of five hours. However, they excreted 600 and 940 mg., respectively, of urine urea nitrogen above the basal excretion. As in unanesthetized

TABLE I

EFFECT OF SODIUM AMYTAL ON BLOOD UREA NITROGEN

DOG NUMBER	DATE	WEIGHT IN KG.	SODIUM AMYTAL ADMINISTERED MG.	BLOOD UREA NITROGEN MG. PER CENT			URINE UREA N. MG. PER HR.
				BEFORE SODIUM AMYTAL	APPROXIMATELY 1½ HR. AFTER SODIUM AMYTAL	APPROXIMATELY 3 HR. AFTER SODIUM AMYTAL	
8	7/12/35	7.7	400	34.4	26.3	23.8	229.8
9	7/16/35	9.5	650	17.9	13.4	11.2	266.5
10	7/18/35	4.5	300	32.1	25.5	20.6	302.6
11	7/25/35	9.1	500	26.2	28.0	25.5	244.7
12	7/30/35	6.4	625	14.6	15.1	13.4	98.4
13	8/ 1/35	7.0	500	12.2	16.5	15.8	36.8
14	9/ 6/35	5.5	400	13.3	10.5	--	83.1
15	9/ 9/35	5.9	500	18.5	--	13.6	93.6
16	9/16/35	5.9	550	16.5	14.8	14.7	87.8
17	9/18/35	5.5	450	26.7	25.3	25.1	111.9
18	9/20/35	6.6	525	16.4	18.6	17.6	106.6
19	9/23/35	7.3	550	16.5	16.2	16.4	66.3
20	9/27/35	9.5	900	10.5	10.5	8.5	115.8
21	9/30/35	6.1	600	10.6	13.4	15.1	49.2
22	10/ 4/35	8.2	800	11.3	11.3	11.3	106.2
23	10/ 7/35	9.0	700	5.6	6.9	7.3	44.0
24	10/11/35	6.8	600	9.1	10.1	13.4	70.2
25	10/14/35	5.7	550	7.6	6.2	9.1	102.8
26	10/15/35	9.1	550	12.1	10.4	--	39.4
27	10/16/35	9.3	600	7.6	7.1	8.5	144.4
28	10/18/35	11.3	750	11.8	10.4	10.7	179.2
29	10/22/35	9.9	650	9.4	7.7	9.5	86.5
30	10/24/35	8.9	550	9.1	9.6	8.6	61.8
31	10/25/35	7.5	600	15.4	8.7	8.1	260.4
32	10/28/35	6.1	450	10.7	10.1	11.1	90.8
33	10/30/35	8.6	650	11.3	7.4	8.3	91.2
34	10/31/35	10.0	700	21.1	20.4	16.8	322.5
35	11/ 5/35	10.7	700	19.0	15.7	14.6	411.6
36	11/ 6/35	8.4	600	--	6.3	4.3	60.2
37	11/ 7/35	6.8	400	14.3	12.0	--	209.9
38	11/ 8/35	6.4	500	6.0	5.5	5.7	75.7
39	11/11/35	6.4	550	8.1	6.5	8.3	81.3
40	11/12/35	13.2	750	6.9	5.0	7.2	184.6
41	11/13/35	5.9	400	12.0	13.1	17.2	77.6
42	11/19/35	6.4	450	7.6	4.5	6.0	68.6
43	11/20/35	6.8	625	27.3	24.5	24.5	87.5
44	11/21/35	10.2	750	3.4	4.1	7.9	29.8
45	11/22/35	5.2	400	5.8	6.4	5.3	17.7
46	12/10/35	6.4	400	12.5	12.5	10.3	91.8
47	12/11/35	8.2	600	17.5	18.9	18.9	126.1
48	12/13/35	8.2	600	5.7	5.8	7.2	32.8
49	12/16/35	7.0	425	6.5	6.5	6.1	100.2
50	12/17/35	6.1	425	5.1	4.6	5.3	56.0
51	12/20/35	8.6	675	9.7	9.3	7.6	189.3
52	12/23/35	6.0	475	6.8	7.4	7.8	49.2
53	12/24/35	5.5	450	11.9	12.7	13.1	68.3
54	12/27/35	7.5	600	7.1	8.8	9.1	66.4
55	12/30/35	6.4	500	8.1	9.8	9.7	56.4
Av.				11.7	11.9	12.0	

dogs there was a distinct elevation of blood urea nitrogen after the administration of glycine. One dog returned to about the initial level, three fell below and two rose slightly.

TABLE II

BLOOD UREA NITROGEN
MG. PER CENT

DOG NUMBER	DATE	WEIGHT IN KG.	SODIUM AMYTAL ADMINISTERED MG.	BEFORE SODIUM AMYTAL	1½ HOURS AFTER SODIUM AMYTAL	3 HOURS AFTER SODIUM AMYTAL	1 HOUR AFTER 10 GM. GLYCINE	2 HOURS AFTER GLYCINE	3 HOURS AFTER GLYCINE	4 HOURS AFTER GLYCINE	5 HOURS AFTER GLYCINE
3	6/21/35	5.9	395	--	--	17.2	20.9	--	21.6	--	18.8
4	6/25/35	8.4	420	--	--	30.0	--	35.7	32.6	--	19.0
28	10/18/35	11.3	750	11.8	10.4	10.7	13.6	13.6	13.0	12.1	8.3
29	10/22/35	9.9	650	9.4	7.7	9.5	10.9	17.0	17.9	20.8	15.2
31	10/25/35	7.5	600	15.4	8.3	8.1	8.3	17.7	14.1	11.0	9.1
32	10/28/35	6.1	450	10.7	10.1	11.1	20.8	24.4	22.8	18.7	16.3

It seems evident from our data that sodium amytal, even when given in large doses, does not seriously impair the ability of the kidney to excrete urea. We believe that the lower urea nitrogen excretion found by some workers is probably due to an inhibition of protein metabolism.

SUMMARY

A study of the blood and urine urea nitrogen of 50 dogs anesthetized with large doses of sodium amytal shows that this anesthetic does not seriously interfere with kidney function as measured by urea excretion.

We wish to express our thanks to Mr. C. L. Dupre and Dr. H. A. Shonle of the Eli Lilly Company for kindly supplying the sodium amytal used in this investigation.

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SPOROTRICHOSIS AMONG VIOLINISTS*

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SEVERAL students in one musical organization were found to have a pustular rash occurring in the submaxillary region.

Investigation revealed several points in common in these cases: (1) These were limited to violin students. (2) These students repeatedly played on each other's violins. Two other members of this organization had played only on their own violins and did not have this eruption.

THE PROBLEM

To prove: First, all cases were caused by the same microorganism. Second, the violin was the vector in transmission.

DESCRIPTION OF LESIONS

The lesions were all similar in appearance and were chronic in nature. Grossly, the area of skin in the submaxillary region in all cases was discolored, varying from a light brown to a reddish brown, and had from one to five small pustules elevating the surface. The pustules appeared yellowish and showed no relationship to the hair in the discolored areas. The location of the lesions pointed to the violin as a vector. Each student stated that the lesions appeared only after beginning to play the violin. The duration of the disease varied from one to seven years, and only one student had received any medication.

TECHNIC OF CULTURING

The technic of taking the cultures and smears is as follows: The infected area was swabbed with alcohol and was allowed to dry. The pustule was opened with a sterile needle. Slight pressure brought a yellowish fibrinous pus to the surface. This was divided. Part was spread on a slide and the rest transferred to culture tubes, using sterile cotton applicators. Other cultures and smears were made from the chin rests of the violins, using sterile applicators moistened in sterile water. All direct smears were stained by Gram's method.

CULTURE MEDIA

The following media were used: Glucose peptone broth, Sabouraud, hay broth, hay agar, cardboard with hay broth.

The Sabouraud media and the glucose peptone broth were the same as is often used in growing fungi. A new medium, hay infusion broth, and hay infusion agar were also used. Since sporotricha are found on dry grasses, grains, etc., the author believed hay infusion should be an excellent nutrient for growing this fungus.

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Received for publication, August 13, 1936.

The method of preparation of hay infusion broth consisted of infusing and boiling 50 gm. of ground alfalfa hay in 300 c.c. of distilled water for fifteen minutes. The hay infusion broth was filtered while hot, through cotton, to remove the larger solids. This broth was brownish in color and was slightly acid in reaction when titrated with N/10 NaOH, using phenolphthalein as indicator. Hay agar slants were prepared by adding 2 per cent agar to hay infusion broth. The hay infusion broth and the hay agar were sterilized at fifteen-pound pressure for twenty minutes.

METHODS OF CULTURE

The usual methods of culturing the material from the lesions were used with Sabouraud, glucose peptone broth, hay broth and hay agar slants. It was found that if material were cultured on moist sterile cardboard in tubes, there was no growth. After three weeks, during which time most of the bacteria present had died, hay broth was added aseptically to the previously inoculated cardboard in the tubes.

A method adapted to studying the growth of a fungus under a microscope is as follows: Two pieces of glass, four by six inches, were selected and cleaned. Several thicknesses of filter paper were cut slightly smaller than the glass plates. A window was cut in the center of the filter paper, leaving a one-inch margin of paper on all sides. The windowed paper was placed between the glass plates and these were sterilized in an electric oven at 160° C. After the plates were sterilized and cooled the upper plate was carefully lifted, sterile melted hay agar was poured on the center of the lower plate within the window opening, using sterile precautions. The top plate was replaced and allowed to cool in the oven. When cool, the upper plate was again lifted and the spores of the fungus were planted on the agar. The plates were placed together and held by rubber bands. The filter paper was moistened with water to prevent the agar from drying. The plates were incubated at 37° C. for forty-eight hours.

FINDINGS

The findings in the smears of pus were as follows:

Case 1.—Gram-negative, straight and branching mycelium with gram-positive spores. A very few streptococci were present. These were not considered of etiologic importance since they were found in this case only.

Case 2.—Mycelium and bipolar spores were found. The bipolar spores were gram-positive at each end and were about three microns in length by one and one-half microns in width. There were also many gram-positive streptobacilli which were considered of secondary importance for the same reason as cited in Case 1.

Case 3.—This student had been opening the pustules before this study began. A pustule was selected that had not been opened previously. The pus obtained was bloody but direct smears showed mycelium, spores, and many red blood cells.

Case 4.—Mycelium and spores found as well as a few bacteria.

Violins.—The direct smears from the violins played by these students all showed gram-negative mycelium, gram-positive spores and many bacteria.

The cultural results were as follows:

In Sabouraud and glucose peptone broth the growths appeared in from two days to one week and were less abundant than in other media. A thin pellicle formed which was light in color when it was young and later became brown.

In hay infusion broth the growth appeared early, and at forty-eight hours a definite tan pellicle had formed. This was composed of mycelium with abundant spores. The color darkened and when a month old was a very dark brown, almost black.

On hay agar slants the growth usually appeared in forty-eight hours as tan velvety elevations upon the surface. These gradually spread, became roughened and changed to dark brown or black in color.

In the cultures on sterile cardboard there was no visible growth in three weeks' incubation at 37° C. On the addition of hay infusion broth a growth appeared within forty-eight hours which was similar to that found in hay infusion broth alone. This method proved a convenient one for the elimination of bacterial growth in cultures of fungi.

The plates, when examined after forty-eight hours' incubation, under low power on the microscope, showed single and branching mycelium with spore formation. This mycelium was broader and more turgid in appearance than any found in stained slides. This is probably due to the shrinkage of the mycelium on dehydration. Growth from the spores was by budding and further growth of mycelium occurred as a budding process from near the terminal ends of the mycelial threads. Spores were produced both laterally and terminally from the mycelium. Spores were arranged singly and did not occur in groups or chains.

Albino rats, injected intradermally and intraperitoneally with suspensions of spores in normal saline, showed no gross lesions after twelve days. When killed, each rat was thoroughly wet with 5 per cent phenol before an opening in the skin was made. All internal organs appeared normal. Smears were made from liver, spleen, and heart blood, as well as cultures from these organs. The smears and cultures from liver and spleen showed characteristic mycelium and spores. No mycelium or spores were found in smears or culture from the heart blood.

CONCLUSION

In growth characteristics, spore formation, morphology, and staining, and in the type of lesions from which all cultures were isolated, this organism is a species of sporotrix.

SUMMARY

1. The mycelium and spores isolated from the submaxillary lesions in a series of violin students and from the chin rests of their violins was a sporotrix.

2. Hay infusion media gave the best results of the five media used.

3. Rats showed no illness, but typical organisms were recovered from the liver and spleen of inoculated animals.

4. The assumption is justified that the chin rests of the violins of infected players served as a vector in the transmission of this disease to others who played upon the same instrument.

Acknowledgment.—I wish to thank the following for their advice, criticism and aid: H. E. Peebles, St. Vincent's Hospital, and T. C. Stephens, Morningside College, Sioux City, Iowa.

THE ACTION OF THE MAMMARY GLAND UPON THE OVARY*

Preliminary Report

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IT HAS been common knowledge that the lactating mammary gland has an inhibitory influence upon ovulation and menstruation. This fact has long been known, both by the medical profession and the laity, and among the latter to such an extent that women who are lactating mothers and who are not desirous of again becoming pregnant will continue nursing their infants for a prolonged period. While these facts are not always true, as many exceptions occur, in many instances they hold good.

It might be appropriate to consider the anatomy of the mature mammary gland, and to review what is generally known concerning its hormonal stimulation as related to its development and the function of lactation. The location of the mammary gland, according to anatomists, is in the pectoral region, extending from the second to the seventh rib, and laterally from the outer border of the sternum to the anterior axillary fold. It varies in size in different individuals, depending largely upon the amount of adipose and glandular elements. The gland is divided into lobes, lobules, and its secretory elements the alveoli; ducts drain the alveoli which empty into intralobular ducts, these in turn drain into interlobular ducts, which empty into intralobar ducts that themselves send the secretory fluid through a terminal lobar duct that ends in the nipple. These latter vary in number from fifteen to twenty openings.

It has been shown experimentally that the development of the mammary gland depends upon the stimulation by estrin from the ovarian follicle. The action of progestin from the corpus luteum is inert in this respect. However, when estrin is primarily injected, followed by the administration of progestin, lobular-alveolar increase has been noted.[1]

During the state of pregnancy, there is a tremendous increase of estrin in the circulation, which reaches its peak just before parturition. During the latter months of gestation the mammary gland increases in all its elements. When delivery takes place, there is a marked sudden drop in the estrin con-

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Aided by a grant from the Alice L. Filbert Fund.

I am indebted to Dr. Sante Piracci of the Italian Drugs Importing Co., 225 Lafayette St., New York City, for supplying the mammary substance used to carry out these experiments.

tent. This, according to various investigators, releases the inhibitory action upon the anterior pituitary hormone and the lactogenic hormone from the anterior pituitary lobe becomes active in the stimulation of the alveoli in the secretion of milk. During the lactation period in the majority of women, menstruation does not take place. This can be explained by the low level of estrin in the circulation, that is inhibited by some antagonistic hormonal action, from the lactating breasts, preventing the maturation of the ovarian follicles. This action varies in intensity in some instances, and it might be assumed that when ovulation, menstruation, and pregnancy occur during the lactating period, the gonadotropic hormone from the anterior pituitary lobe overcomes the inhibitory action of the mammary gland.

Upon this conjectural and apparently physiologic basis, mammary gland extracts have been successfully used clinically for a number of years in patients suffering from different types of profuse menstrual bleeding.

These clinical results seemed to warrant laboratory investigation, working from a practical point of view to an experimental explanation of how this action takes place.

LABORATORY INVESTIGATION

In order to have a theory upon which to base the subsequent description of these experiments, it was assumed that there was set free from the breasts at the beginning of lactation a substance that inhibited the maturation of the ovarian follicle and ovulation. In consequence, the estrin production was either limited or entirely inhibited.

These experiments were conducted upon the albino rat, twenty-one days old, weighing 25 to 30 gm. In order to prove follicular activity, the ovaries were stimulated with several gonadotropic substances. The extracts used for this purpose were (1) placenta (A.P.L.), (2) pregnancy urine extracts, and (3) anterior pituitary extracts from cattle. Early in the preliminary period of these experiments it was found that the extracts from (1) and (3) did not give constant gonadotropic reactions, such as typical corpora hemorrhagica, as were found in the administration of pregnancy urine extract. The conclusive portion of these experiments was therefore done with the latter substance.

The placenta extracts injected were prepared according to the method of Collip.[2] The pregnancy urine was collected about the fifth month of gestation, which is the highest level of prolan, and the method of extraction was carried out according to that of Zondek[3] by alcohol precipitation. The anterior hypophyseal lobe extract was prepared by the method outlined by Munch in *Bioassay*.[4]

Following the preparation of these extracts they were assayed by injection into the immature rat and standardized so that 0.6 c.c. contained approximately 1 R. U.

The extracts from the mammary gland that were utilized to inhibit the follicular development were (1) lactating mammary gland extracts, (2) extract made from desiccated mammary gland, and (3) extract from lactating mammary gland prepared by the Istituto Sieroterapico of Milan, Italy.[5] Mammary gland

Extract 1 was prepared in the laboratory by acetone extraction and so concentrated that 1 c.c. represented 250 gm. of the raw gland. Extract from the desiccated mammary gland (No. 2) was prepared by alcoholic extraction but when tested against the gonadotropic stimulant was found inert, and was therefore discarded. The tests were carried out with the mammary extract (No. 1) made by us, and the Italian mammary extract (No. 3) that represented 0.57 gm. of the fresh lactating gland to the cubic centimeter.

INJECTION METHOD

As a control, groups of six animals each were injected with 0.1 c.c. of the gonadotropic stimulant three times a day for two days, after it had been standardized to contain 1 R. U. to 0.6 c.c. In the gonadotropic controls there resulted

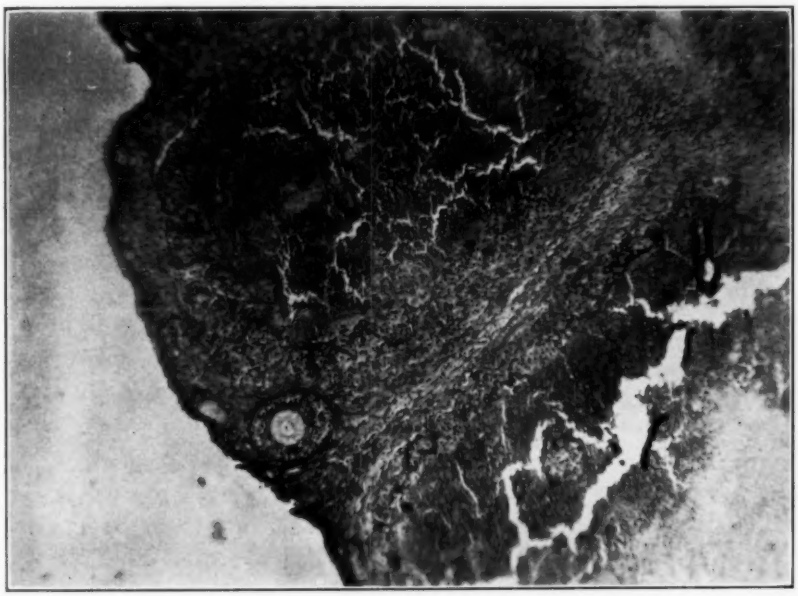

Fig. 1.—Gonadotropic control. Microscopic section of ovary of immature albino rat, following injection of 1 R.U. of gonadotropic hormone (pregnancy urine extract). Note corpora hemorrhagica and lutein formation.

in every instance corpora hemorrhagica and corpora lutea. In the mammary control there was no change noticed either macroscopically or in section. The experimental animals were given 1 R. U. of gonadotropic substance over a period of two days, and in addition 1.2 c.c. of mammary extract were injected at the same time. These substances were mixed in the same syringe and administered subcutaneously in the back. The animals were sacrificed ninety-six to one hundred hours following the first injection. The ovaries were dissected and removed for microscopic section and study (Figs. 1, 2, and 3).

RESULTS

In most instances there was a complete absence of corpora hemorrhagica and corpora lutea. Follicular development was present in the majority of instances, but in some animals there was an absence of this development as was

Fig. 2.—Mammary control. Microscopic section of ovary of immature albino rat, following injection of 1.2 c.c. of mammary substance. Note predominance of primordial follicles and a few showing slight development.

Fig. 3.—Injection of gonadotropic hormone and mammary substance. Microscopic section of immature rat ovary, following injection of 1 R.U. of gonadotropic hormone and 1.2 c.c. of lactating mammary gland extract. Note absence of corpora hemorrhagica and lutea. A few follicles show slight development.

confirmed by the negative vaginal smears, and microscopic examination of sections. It is of interest to note that the recent work of Kim[6] has shown similar findings.

LABORATORY EXPERIMENTS ON RABBITS

A series of rabbits were given an aqueous extract of placenta, made from the desiccated substance. Ten cubic centimeters were administered daily for three days by the oral and intravenous routes. When the animals were sacrificed on the fifth day, typical corpora hemorrhagica and corpora lutea formations were obtained in both instances. The minimum amount of placenta given by mouth that gave a typical Aschheim-Zondek reaction was 8 c.c.

Another series of rabbits were given, by mouth and intravenously, ten c.c. of mammary extract for three days and when sacrificed upon the fifth day, no microscopic change in the follicular mechanism was noted.

Following the establishment of the above controls, a third series of rabbits was given equal amounts (10 c.c.) of mammary and placental extracts for three days. On the fifth day the animals were sacrificed and in more than 90 per cent of the ovaries, there was a complete absence of corpora lutea as observed on microscopic section. Some of the ovaries showed the follicles solely in the primordial stage, but the majority of the follicles showed moderate development. The inhibitory effect of the mammary extract was apparently as effective by mouth as intravenously, and evidently the active substance was not destroyed by the gastric juices.[7]

These experiments prove that there is a definite inhibitory action of the mammary upon the ovary. Further laboratory work upon the rat and rabbit is now being done, in a larger series, in order to substantiate these findings.

It is also worth while to mention that early in these experiments, when the inhibitory action upon the follicle was noted, it was thought possible to be able to find a basis for the standardization of mammary gland extracts, as an anti-estrin factor. Roughly speaking, a mammary rat unit can be defined as the minimum amount of mammary extract which inhibits follicular development, following the injection of 1 R. U. of gonadotropic hormone.

CLINICAL RESULTS IN MAMMARY GLAND THERAPY IN MENORRHAGIAS

Menorrhagia, or essential uterine hemorrhage of endocrine origin, can be grouped as occurring in (1) the adolescent state, (2) during active sexual life, and (3) during the menopause.

The outstanding cause irrespective of grouping is an overproduction of estrin. To attack this problem a definite diagnosis must be made. A complete physical examination should be done, in order to eliminate blood dyscrasias, anemias, and leucemias, as well as a thorough pelvic examination to eliminate adnexal infections, uterine and ovarian tumors, and cervical pathology. The examination should then be concluded by an endometrial biopsy, to exclude the possibility of fundal carcinoma. All endometrial biopsies obtained from patients who have uterine hemorrhages of glandular origin show a typical hyperplasia.

A series of fifty patients who had menorrhagia of glandular origin were placed upon mammary therapy. The adolescent group is not included in this series because of therapy other than glandular being administered. This discussion will be limited solely to the menorrhagias of women who were in the childbearing period of life and those approaching the menopause. There were 32 patients in the first group and 18 in the second group. The youngest patient was twenty-three years old, and the oldest was forty-nine years. Owing to such a small series of cases, no attempt will be made to give a detailed statistical report. All patients can be subdivided, according to the amount of blood lost, into moderate and severe groups. The majority of patients were in the moderate class where the menstrual blood loss incapacitated them from business or social affairs to only a slight degree, while those in the severe group had a blood loss during menstruation that seriously interfered with their duties of life and continually produced a state of secondary anemias of varying degrees. Estrin determinations made upon some of these patients showed a definite increase. In applying mammary therapy, it was discovered quite early that the desiccated powder in the form of tablets or capsules had very little or no effect, irrespective of the dosage given. This was discontinued and therapeutic measures were confined to two types of medication, a liquid preparation made from the desiccated powder representing 1 gr. to 5 minims, which was administered both orally and intramuscularly. The other preparation was a product obtained from the Istituto Sieroterapico Milanese, extracted from the raw lactating gland in the proportion of 0.57 gm. (9 gr.) to the cubic centimeter, and was administered solely by intramuscular injections.

With very few exceptions, all patients showed improvement in the diminution of the excessive menstrual flow, and were able to carry on their usual mode of life without difficulty. In the severer forms, where the patients were seriously incapacitated from the loss of blood, associated with a marked anemia, there was prompt improvement of the physical state when the blood loss was lessened by mammary gland therapy. In several instances the anemia was such that blood transfusions were necessary to initiate a recovery. Blood transfusions in these severe types do not perpetuate a cure, as the condition recurs if not supplemented by appropriate glandular therapy.

In the more moderate forms of menorrhagia, a fairly prompt response to therapy was noted. The majority of the patients reacted to oral medication. In the severe forms, supplementary injections were necessary to obtain a cessation of the flow.

In comparing the efficacy of preparations, it is quite definite that a more rapid result is obtained from the raw lactating preparations than that made from the desiccated powder. The former, while containing less actual tissue, is more potent in smaller quantities (1 to 2 c.c. doses) than the latter (5 to 10 c.c.). This is a distinct advantage in many ways, especially when one considers the discomfort to the patient following 5 to 10 c.c. injections. Also one cannot help but feel that the lactating gland has a more powerful action upon the inhibition of the growth and development of the follicular mechanism, restraining the excess of estrin production. Further experimental work is now being done with the nonlactating gland to ascertain what difference may exist

in this respect. In this connection it should be mentioned that Cronental,[8] by stimulating the mammary gland with galvanism, in patients suffering from menorrhagia, was able to report improvement in 86 per cent of instances.

The application of mammary therapy in menorrhagic conditions should be of a tentative nature. It is recommended that one must feel one's way, as the individual response is different. Otherwise serious and perhaps permanent damage may be done to the follicular apparatus, producing complete ovarian inhibition. This is well illustrated physiologically in the so-called lactational amenorrheas, followed by atrophy of the uterus, among patients who for various reasons continue nursing their infants over a prolonged period. Quite recently, a similar condition resulted by administering large doses of mammary extract to a menorrhagic patient of the severe type, where a temporary amenorrhea was inadvertently brought about with the usual menopausal syndrome of flushes, sweats, nervous irritability, etc. In this instance, it was necessary to give replacement therapy in the form of estrin to overcome the excessive inhibition of the mammary substance.

SUMMARY

Menorrhagias of different forms seem, from the foregoing experimental evidence, to be due to an excessive amount of estrin. The mechanism of this overproduction may be a direct action of the gonadotropic hormone from the anterior pituitary lobe, producing an imbalance between the corpus luteum hormone and that of the follicle. The experiments upon the female rabbit and rat stimulated by the gonadotropic hormone with simultaneous injections of mammary extracts showed a definite inhibition and retardation of follicular maturation with decreased production of estrin. Clinical results in menorrhagia of glandular origin treated with mammary therapy would indicate that its action upon the ovary was one of restraint in the manufacture of the estrogenic hormone. When applied in a careful and judicious manner, mammary therapy seems to be selective for these conditions.

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LABORATORY METHODS

CALIBRATION OF MICROPIPETTES AND TUBES SIMPLIFIED*

LILLIAS D. FRANCIS, PH.D., NEW HAVEN, CONN.

QUANTITIES of blood much less than 0.01 ml. may be taken from small animals with accuracy sufficient for exacting microbiochemical methods by the use of hemocytometers; but such pipettes, designed as diluting devices, are highly inaccurate for volumetric measurements unless carefully calibrated. Indeed, calibration of twelve blood diluting pipettes used for counting white corpuscles showed that the actual capacity to a given graduation mark varied widely, the volume of one hematimeter was almost three times that of another calibrated to the same mark. Moreover, variations in the volumes of the bore were such that to secure 0.0156 ml. of blood, one pipette was filled to line 6, another to line 7, and a third to line 10.

Calibration of such small pipettes and Newcomer hemoglobinometers by the usual gravimetric method can be accomplished with great precision by using mercury if the difficulty of its rapid movement can be regulated adequately. A device is illustrated by means of which its control may be achieved easily. By suitable manipulation of this apparatus, mercury can be made to enter a capillary tube slowly, then the column may be moved at will and halted at any desired point with little difficulty.

The figure shows a bed of mercury, *M;* a hemocytometer, *H*, supported at an angle of about 10 degrees by the use of two levels, *a* and *b;* and a 5 ml. glass syringe, *S*, connected with the pipette, *H*, through a capillary tube, *C.T.* By means of a magnifying glass, *M.G.*, supported above the pipette, movement of mercury within the capillary bore of the hematimeter could be seen in such detail that control was possible with greater exactness than was secured without such visual aid.

Glass tubing having an outer diameter of 3.3 mm. and a bore 1.0 mm. in diameter was used to make the capillary tube. The best results were obtained from a capillary 24 cm. in length, 0.38 mm. in outer diameter, and having a minimum internal diameter of 0.05 mm. The size of this microtube could not be varied much from these dimensions because, if the bore were measurably greater, the shifting of the mercury could not be controlled with sufficient precision; the slightest movement of the syringe caused so much suction or push that the position of the column of mercury changed rapidly. On the other hand, if the capillary tube had a bore much less than specified, the effect of suction upon the bed was insufficient to draw mercury into the micropipette and, moreover, the capillary then broke frequently.

*From the Department of Physiological Chemistry, Yale University.
Received for publication, April 27, 1936.
Aided by a grant from the Josiah H. Macy, Jr., Foundation.

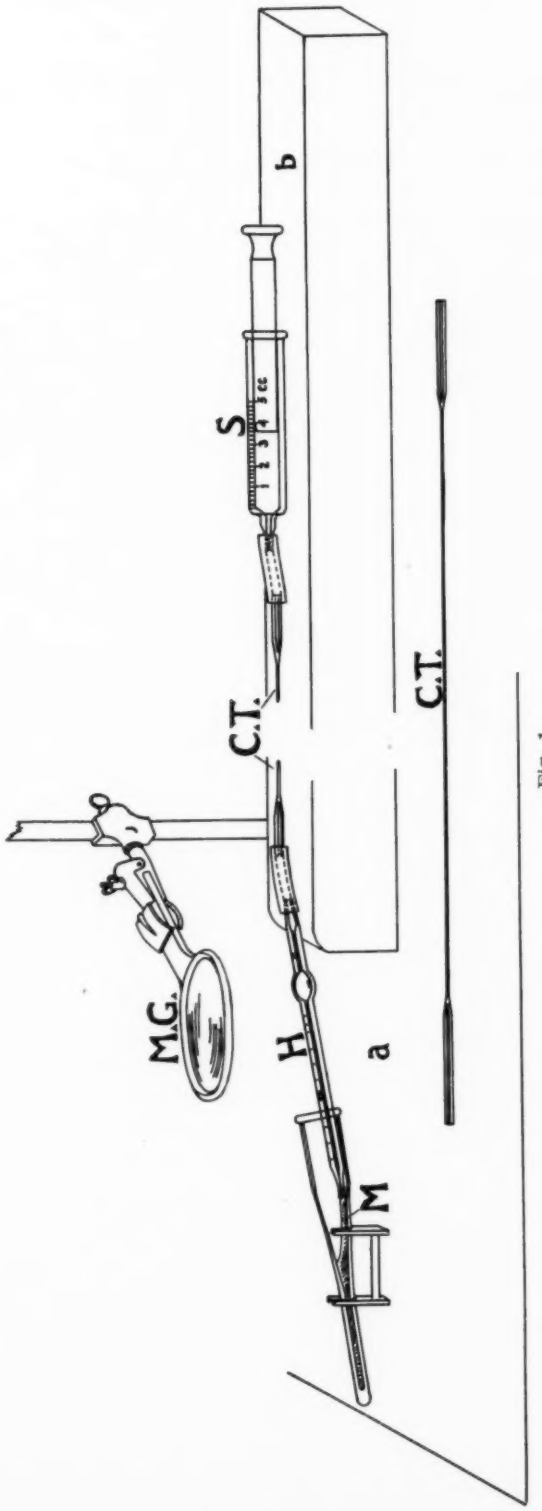

Fig. 1.

In operation one hand controlled the hemocytometer and the other the syringe. When mercury had reached a desired mark or had been adjusted to it, the tip of the pipette was removed quickly from the supply of mercury; the measurement was checked if necessary, and then the loaded, tared hematimeter was weighed. In order to fill the tube to mark 11, above the bulb, the angle of support was increased beyond 10 degrees by lowering the bed of mercury.

In attempting to calibrate specially made microcentrifuge tubes, one of which is pictured, it was found that the narrowness of the bore prevented mercury from sinking by gravity or even being shaken to the bottom of the tubes. In order to measure accurately the capacity of such tubes to 0.001 ml., the apparatus described was modified slightly in form. The capillary tube used had only one end of undrawn glass, and by this end it was attached to a syringe; the other terminus was of capillary size; hence the tube was used as a micropipette controlled through the attached syringe. Mercury, picked up by this microtube, was transferred to the centrifuge tube in such a manner as to avoid trapping air below the layer of mercury. Examination for air bubbles was made by the aid of a magnifying glass; if necessary, the mercury filling was shaken down and prodded by a glass rod of minute diameter. By the use of a capillary pipette in the manner described, it became an easy matter to deliver mercury into the centrifuge tube, or remove it in microquantities.

The device described has made possible the calibration by mercury of the three kinds of capillary glassware mentioned. With suitable modifications it is applicable for use with other tubes and pipettes of minute bore which are difficult to fill with mercury for calibration by the gravimetric method.

The writer wishes to express appreciation and thanks to the artist, Mr. P. Frithiof Nelson, for his excellent illustration of the assembled apparatus.

THE GROWTH OF BACTERIA FROM THE BLOOD STREAM IN DEFIBRINATED BLOOD*

ANNA DEAN DULANEY, PH.D., AND FRANCES GUTHRIE, B.A., MEMPHIS, TENN.

AN ACCEPTED teaching in bacteriology is that bloods for cultures should be diluted five to ten times with broth in order to minimize the bactericidal effects of the serum. Shaw[1] has recently refuted this idea and reports the isolation of various bacteria from the blood by incubating the defibrinated blood of the patient. He found that this method yielded even higher percentages of positive cultures than those obtained by inoculation of the blood to media of suitable types.

In this paper a report is made of a series of blood cultures comparing Shaw's technic with one routinely employed in our laboratory. On 175 unselected hospital patients where blood cultures had been requested by the clinical staff, duplicate cultures were run. For comparison with the defibrinated blood we chose brain medium which has been found to yield the highest percentage of positive cultures in routine work. This medium is prepared by adding dextrose veal infusion broth, containing a phosphate buffer, to macerated brain, tall tubes of approximately 100 c.c. capacity being used.

We followed Shaw's directions and simply delivered 10 c.c. of patient's blood to an Erlenmeyer flask of 50 c.c. capacity, containing a small quantity of glass beads. Ten cubic centimeters of blood were also inoculated to brain medium. All cultures were incubated at 37.5° C. and examined by smears and subcultures at the usual one-, two-, and five-day time periods. When typhoid fever was suspected, we drew an additional quantity, 5 c.c., of blood for agglutination tests.

TABLE I

ISOLATION OF BACTERIA FROM BLOOD STREAM BY USE OF DEFIBRINATED BLOOD AND BRAIN MEDIUM

MEDIUM	TOTAL NUMBER CULTURES	RESULTS		ORGANISMS ISOLATED					
		POSITIVE	NEGATIVE	EB. TYPHOSA	S. PARATYPHI B.	E. COLI	STREP. HEMOLYTICUS	PNEUMOCOCCI	STAPH. AUREUS
Defibrinated blood	175	35	140	13	1	4	2	14	1
Brain	175	34	141	13	1	3	2	14	1

Table I gives the results obtained. It is seen that the defibrinated blood method yielded positive results in every case where organisms were demonstrated in brain medium, and in one instance where the brain medium remained negative. It is significant that diversified types of bacteria were isolated under such conditions. The organisms grew luxuriantly and were readily identified.

*From the Department of Bacteriology, University of Tennessee, Pathological Institute. Received for publication, May 13, 1936.

Tests for H and O agglutinins were run on sera from 9 patients with typhoid fever. The results were as follows:

SERUM	AGGLUTININ TITER	
	H	O
1	640	2560
2	640	2560
3	>20	2560
4	1280	2560
5	>20	320
6	2560	2560
7	>20	80
8	1280	1280
9	320	640

It is readily seen that sera containing a high concentration of O agglutinins were not inhibitory to growth of the organisms, at least in the undiluted state existing in defibrinated blood.

In four cases we obtained positive cultures in brain medium when less than 5 c.c. of blood could be obtained from the patient. These cultures are not included in the table because no defibrinated duplicates were run. When only small amounts of blood can be secured by routine methods it would seem better to inoculate this to suitable media because of the difficulty in making smears and subcultures.

We found it necessary to use rubber stoppers in the flasks to prevent excessive evaporation, a detail not mentioned by Shaw.

The only tedious part of the procedure is cleaning of the glass beads. Potassium bichromate sulphuric acid cleaning solution readily frees them of blood, but this solution must be thoroughly removed, preferably by rinsing in running water overnight.

The defibrinated blood method is a very cheap and simple one and recommends itself especially to the small laboratory where blood cultures are not often requested and such media not kept ready for use. Under most conditions it is equally as satisfactory as inoculation of whole blood or clot to culture medium.

CONCLUSIONS

1. The dilution factor does not seem to have the importance in blood culture work usually accorded it.

2. Pathogenic microorganisms grow luxuriantly in the defibrinated blood of the patient.

3. A high antibody content of serum (in typhoid fever) does not inhibit growth.

We are indebted to Dr. J. E. Kite and Dr. J. M. McCulloch for assistance in collecting and examining part of the cultures.

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A NOTE ON MANTLES FOR BERKEFELD AND MANDLER FILTERS*

S. Brandt Rose, M.D., Philadelphia, Pa.

THE mantle ordinarily supplied for Berkefeld and Mandler filters requires a comparatively large volume of liquid to cover the cylinder completely. Although a smaller amount of fluid will suffice when a test tube is inverted over the filter, the use of this test tube sometimes presents technical difficulties.

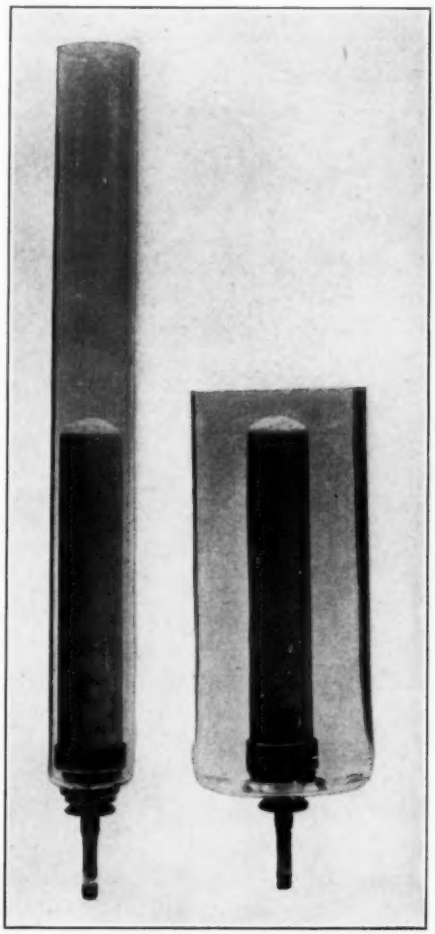

Fig. 1.

A narrow mantle is suggested that obviates the need for the inverted test tube because the diameter of this mantle is only slightly larger (3 to 5 mm.)

*From the Division of Bacteriology, Laboratories of the Philadelphia General Hospital.
Received for publication, June 25, 1936.
†Available in Pyrex glass from Arthur H. Thomas Company. Philadelphia, Pa.

than the filter cylinder itself. An adequate capacity is obtained by making the mantles longer than the regular ones. The figure shows a 5 by 1 filter in both types of mantle.

The volume of liquid needed to cover different sized cylinders when the regular and narrow mantles are used, is contrasted in Table I.

TABLE I

FILTER CYLINDER	REGULAR MANTLE	NARROW MANTLE
1⅜ × ⅝	16 c.c.	4 c.c.
2½ × ⅝	28 c.c.	6 c.c.
2 × 1	185 c.c.	13 c.c.
5 × 1	360 c.c.	25 c.c.
8 × 1	545 c.c.	34 c.c.
10 × 2	1,700 c.c.	110 c.c.

The data in Table I are approximate because the cylinders and mantles vary somewhat in size. The narrow mantles† have been in use for over two years and have proved convenient for the filtration of relatively small amounts of material (bacterial filtrates, serums, etc.).

A SIMPLE MECHANICAL DEVICE FOR SHAKING BLOOD CELL COUNTING PIPETTES*

CARL OLSON,* D.V.M., ROCHESTER, MINN.

VARIOUS devices are available for shaking blood cell counting pipettes, two principal advantages gained by the use of such devices being: (1) thorough mixing of the blood cells, and (2) more rapid work on the part of the technician doing the counting. The devices offered on the market, however, are relatively expensive and perhaps for that reason are not widely used.

The use of the vibrating armature of the ordinary electric doorbell as a shaking mechanism was suggested by Putnam. As certain modifications have been made in the construction of the apparatus and many years have passed since it was originally described, it seemed worth while to describe this serviceable piece of apparatus again.

An ordinary electric doorbell may be purchased for approximately 75 cents. The gong is removed and the angular projection of the base supporting the gong is flattened or broken off. The end of the armature bearing the ball is cut off and the remaining portion is straightened. A short portion of the end is then bent in a horizontal plane, at right angles to its length. The exact location of this bend depends on the construction of the particular doorbell and its rate of vibration. If the bend is situated too far from the axis,

*From the Department of Pathology and Bacteriology, New York State Veterinary College at Cornell University, Ithaca, New York. Work done at the Institute of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.

Received for publication, July 30, 1936.

the electromagnet will not be of sufficient strength to impart a rapid rate of vibration; conversely, if it is situated too near, the rate of vibration will be too rapid and the movement will be of insufficient amplitude. The movement may be adjusted to some extent by bending the "make and break" post, either toward or away from the point of contact with the armature. This adjustment should be made after the device is assembled and a blood pipette is in position.

The pipette holder is made of a narrow bar of brass. Near one end of this bar is a bend, forming a recess into which the bulb of the pipette fits. Two U-shaped strips of copper are soldered at right angles to each end of the brass bar. These are fastened by one end of the "U" with the open end

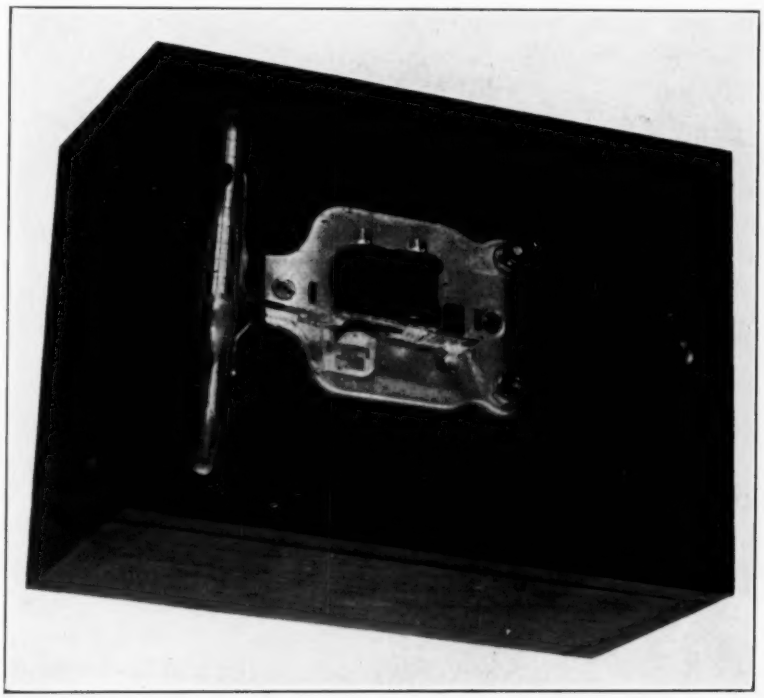

Fig. 1.—Hemocytometer pipette shaking mechanism with pipette in place. The cover has been removed from the doorbell to show the sites of reinforcement of the armature. A small electric switch, of the chain-pull type, may be noted at one end of the box cover.

uppermost and the free arm away from the magnets, as may be seen in Fig. 1. The holder is soldered to the bent end of the armature.

A wooden box is constructed to house snugly two ordinary 1½ volt dry cells. The cover is fastened to the box with screws so it can be removed. A small electric switch of any type and the modified doorbell are fitted in the cover. It may be necessary to construct the box slightly longer than the batteries in order to provide clearance for the switch. If this is done, the batteries can be secured in place by a wooden cross-member placed inside the box.

The dry cells may be wired either in series or parallel. It is believed that parallel wiring results in greater life of the batteries. Batteries have withstood months of heavy use without having to be replaced when wired parallel.

It has been customary, in my work, to fit a heavy rubber band over the ends of a blood pipette as soon as a sample is obtained. The principal reason for this procedure is to prevent evaporation of the fluid content. The rubber bands are cross-sectional strips, cut from an ordinary automobile inner tube, 3½ inches in diameter. The blood pipette with its rubber band is placed in the pipette holder in a horizontal position (Fig. 1). The loose ends of the copper strips are pinched against the flat side of the rubber band. This gives a firm grasp on the pipette and prevents it from being shaken loose.

The box and batteries are of sufficient weight to hold the apparatus in place while the device is being operated. The entire piece of apparatus is of such size that it may be conveniently moved from place to place.

Certain parts of the ordinary doorbell will require reinforcement, which can be done with solder and short pieces of stiff wire, or with solder alone. The place where the armature becomes narrow is one point that may require additional strength. The attachment of the thin brass strip (which bears the movable contact point) to the armature may also have to be strengthened. After considerable use, the armature is likely to break loose from its fastening. When this occurs, the pipette holder can be removed and fitted to a new doorbell.

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A SIMPLE METHOD FOR OBTAINING EFFECTIVE ANAEROBIOSIS*

JAMES E. WEISS, PH.D., AND EARLE H. SPAULDING, PH.D., NEW HAVEN, CONN.

AN IDEAL method for providing bacterial cultures with an oxygen-free environment must be simple, rapid, efficient, inexpensive, and at the same time safe for the operator. None of the procedures in use at the present time possesses all of these qualities. A few years ago the authors needed a more satisfactory technic in their studies of two different groups of nonsporulating obligate anaerobes, namely the Fusobacterium and the Bacteroides genera. The difficulty lay in securing and maintaining strictly anaerobic conditions for a week or more and at the same time preventing excessive dehydration of the media.

Strict anaerobiosis is much more easily achieved in deep agar or deep liquid media than on agar plates. Nevertheless, surface growth is, as a rule, more desirable for the isolation and identification of anaerobes; in fact, it is in some instances the only practical method. The technic to be described here has proved to be highly satisfactory for surface colony production and study.

The procedure used at first consisted in the mechanical removal of the oxygen by alternately evacuating and rinsing the culture jars with an inert

*From the Department of Bacteriology, Yale University.
Received for publication, July 29, 1936.

gas, the final evacuation being as nearly complete as possible. This method was undesirable because either the jars leaked or the agar plates, after two days in vacuo, became so thoroughly dehydrated that they were useless. Furthermore, the vacuum caused much of the escaping moisture to collect on the free agar surface and thus rendered the plates liable to contamination.

The technic now employed by us is not based upon any new device or principle, but is merely a combination of several methods. The procedure is so simple that it can be used not only in specialized research, but also in classroom and laboratory demonstrations.

McIntosh and Fildes (1916) made use of a method in which the removal of oxygen is accomplished by the oxidation of admitted hydrogen under the influence of palladinized asbestos wool. This catalyst, as described by Zinsser and Bayne-Jones (1934), is packed or rolled against wire gauze and heated electrically to initiate the catalytic process. Such a device is expensive, occupies valuable space within the jar and subjects the operator to the danger of explosion.

Eggerth and Gagnon (1933) employed a modified form of the catalyst. When it is more or less finely divided, palladinized asbestos is sufficiently active to render heating unnecessary. Thus, the cumbersome electrical chamber is avoided, and the possibility of explosion somewhat reduced. Although the technic is a distinct improvement over the simple evacuation procedure, considerable time is still required for the process. For absolute safety, the hydrogen must be passed into the jar very slowly, the operation requiring about fifteen minutes. Furthermore, no effort is made to remove the dissolved oxygen.

Boëz (1927) conceived the idea of evacuating the jar prior to the addition of the hydrogen. The gas could then be added much more rapidly, since only a small amount of oxygen remained in the jar. Nevertheless, as this author continued to use a relatively inactive form of the catalyst, the expensive electrical chamber was still required.

By simply combining the improvements suggested above, an almost ideal anaerobic technic is obtained. A small, flat porcelain dish containing the catalyst (palladinized asbestos) is placed in the jar the ground surfaces of which are coated with vaseline or, better still, Cello-Seal (Fisher). No other seals are necessary. The jar is connected by means of a three-way stopcock with a system containing a Cenco-Hyvac pump, manometer and hydrogen tank. The jar is then evacuated to the negative pressure desired, and the pump disconnected by the stopcock. Hydrogen is passed into the jar until atmospheric pressure is restored. A jar containing inverted Petri plates can be evacuated safely to a negative pressure of 720 mm. of mercury, while with liquid media a manometer reading of 740 mm. is usually reached before bubbling begins.

No special or intricate jar is needed. Hempel desiccators having a capacity of ten standard Petri plates or forty 5/8 inch tubes are completely satisfactory. Four-tenths of a gram of the catalyst is sufficient to establish anaerobiosis in this type of jar, as shown by the reduction of methylene blue. The indicator

is prepared by adding the dye in a final concentration of 1:50,000 to 1.0 per cent dextrose extract broth, pH 7.2. The blue color disappears in from six to eight hours under anaerobic conditions.

In the cultivation of organisms which are stimulated by added carbon dioxide, the desired amount of the gas may be introduced into each jar before the addition of hydrogen, simply by connecting the CO_2 tank to the system.

The procedure described here is safe, clean, rapid, and efficient. The quantity of oxygen remaining in the jar after evacuation is so small that danger of explosion is eliminated. Five minutes is the average time consumed in placing forty culture tubes or ten Petri plates under anaerobic conditions. The agar plates can be inverted with safety, since complete evacuation is not necessary. There is no tendency toward dehydration of the solid medium, even after three weeks' incubation; indeed, there is much less drying than under ordinary aerobic conditions. This method is also quite applicable to the study of anaerobic fermentation, for, although complete and constant anaerobiosis is obtained, the evacuation can be stopped before the liquid is drawn out of the gas tube.

The catalyst gradually loses its efficiency through adsorption of moisture. If, upon removal from the jar, it is heated for a few minutes over a Bunsen burner, its full activity is restored.

In this laboratory the method has been used with complete success by individuals and by different classes in growing the common spore-forming anaerobes, both on solid and in liquid media. It has also been employed in isolating and studying certain groups of anaerobic nonsporulating bacteria which require long incubation; in fact, such studies could hardly have been made without the use of a similarly efficient and practical method.

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THE NEW VAN DEN BERGH REACTION FOR THE DETERMINATION OF SERUM BILIRUBIN UTILIZING THE PHOTELOMETER*

ARNOLD E. OSTERBERG, PH.D., ROCHESTER, MINN.

THE original van den Bergh reaction applied to the determination of serum bilirubin as it has been modified particularly by Thannhauser is used extensively as a clinical test for the determination of hepatic damage and function. However, its many well-known inherent errors lessen to some degree the quantitative aspects of the reaction. Among these may be mentioned factors responsible for loss of color from absorption on the protein precipitate and the necessity for using artificial standards for comparison. Errors attributable to absorption of color in serums having a high bilirubin content can be partially overcome by utilization of the procedure involving dilution of the original serum before the van den Bergh reaction is applied. Inasmuch as the direct reaction masks the indirect reaction, it is not possible to determine that portion of the total bilirubin which will produce an indirect reaction and that portion which will produce a direct reaction.

With the purpose of eliminating several of these errors, van den Bergh and Grotepass recently have improved the technic and have made color comparisons using a dimming wire gauze illuminated by monochromatic light; the instrument is standardized previously with azobilirubin derived from purified bilirubin. I have adapted this technic to the use of the photelometer, using a Wratten No. 74 filter having a maximal transmission at 530 to 535 millimicrons, according to the technic described by Sheard, Sanford and Osterberg. In this manner the use of an ether solution of iron thiocyanate or an artificial standard of cobalt sulphate or of permanganate is obviated and direct comparison and calibration can be determined with purified bilirubin converted to its azo derivative.

Obviously, calibration curves must be determined for any particular photelometer using a purified bilirubin. As van den Bergh and Grotepass have shown, lots of artificial, purified bilirubin from several different sources yield the same result.

The calibration curve is obtained by the following procedure: Dissolve 5 mg. of purified bilirubin in 100 c.c. of chloroform and evaporate 10 c.c. of this solution under carbon dioxide on a water-bath, until it is nearly dry. To the residue add 80 c.c. of a solution consisting of 50 per cent alcohol which contains 10 c.c. of a buffer solution, described later, of pH 6.6 to every 100 c.c. The buffer alcohol solution is heated for some time in order to evaporate the chloroform. Next the solution is brought to room temperature and to it is added 20 c.c. of the reagent consisting of (A) 1 gm. sulphanilic acid, 15 c.c. of 25 per cent hydrochloric acid with distilled water up to 1 liter, and (B)

*From the Section on Clinical Biochemistry, the Mayo Clinic.
Received for publication, June 26, 1936.

0.5 per cent sodium nitrite in aqueous solution. Ten cubic centimeters of Solution A is mixed with 0.3 c.c. of Solution B shortly before use. The buffer containing alcohol is added to the solution after the reagent and the total quantity has been brought up to 100 c.c. The pigment solution which is to be examined must be left in the dark for fifteen minutes in order to complete the "coupling."

This concentration of bilirubin as its azo derivative equals 1/200,000 or 0.5 mg. in 100 c.c. This corresponds to a concentration of bilirubin of 5 mg. in each 100 c.c. of serum when the analysis is carried out on 1 c.c. of serum and the final volume equals 10 c.c.

Other points on the curve are obtained in a similar manner using volumes of the original bilirubin solution of 8 c.c., 6 c.c., 4 c.c., and 2 c.c. By this pro-

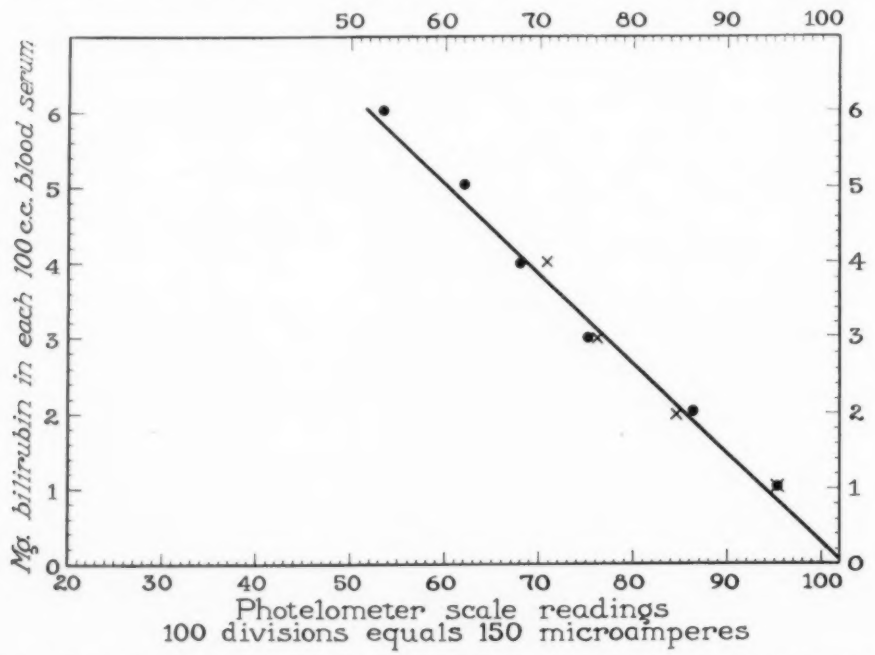

Fig. 1.—The relationship between milligrams of bilirubin in each 100 c.c. of blood serum and photelometer readings.

cedure, as is shown in Fig. 1, there is obtained, for practical purposes, a straight line relationship* up to values corresponding to a concentration of 5 mg. of bilirubin in each 100 c.c. of serum. If higher concentrations than this value are obtained, it is necessary to dilute the serum previous to the determination in order to obtain a value below 5 mg. in each 100 c.c. Appropriate factors are then applied to obtain the final value of bilirubin. This procedure is adopted because of the fact than when values above 5 mg. per 100 c.c. are subjected to the diazo reaction the photelometric readings depart too greatly from the assumed straight line relationship. Also, the concen-

*It is known that, in the case of solutions of substances which possess characteristic absorption zones, the concentration (C) is proportional to the negative logarithm of the transmitted light (I); that is, $C = -k \log I$. However, for the range of dilutions used in these investigations, departures from the linear relationship as shown in Fig. 1 and the values obtained from the semilogarithmic plot are not considered as being significant.

tration of the colored compound produces a very dark solution and turbidity may develop. The technic that has been applied is similar to that of van den Bergh and Grotepass and is described next.

TECHNIC

Reagents.—The diazotizing reagent is prepared by adding 0.3 c.c. of Solution B to 10 c.c. of Solution A and is freshly prepared.

Solution A: To 0.5 gm. of sulphanilic acid are added 10.4 c.c. of 50 per cent hydrochloric acid, and the volume is made up to 500 c.c.

Solution B: 0.5 per cent sodium nitrite solution.

The alcoholic buffer solution. Fifty c.c. of Sorenson's phosphate buffer solution of pH 6.6 is made up to 250 c.c. by the addition of alcohol. Water is then added to make the volume 500 c.c.

Sorenson's buffer solution of pH 6.6 is prepared by adding 40 c.c. of fifteenth molar disodium acid phosphate ($Na_2HPO_4.2H_2O$) to 60 c.c. of fifteenth molar potassium dihydrogen phosphate (KH_2PO_4).

Direct Reaction.—To 2 c.c. of reagent in a graduated centrifuge tube 1 c.c. of serum is added and the mixture is allowed to stand five minutes. Add 2 c.c. of water and alcohol buffer solution to the 10 c.c. mark. Allow to stand fifteen minutes and read the amount of color developed in the photelometer. If the reading is below 50 on the scale, the serum is diluted with salt solution and the determination is repeated until the reading on the microammeter is more than 50 divisions on the scale.

Indirect Reaction.—To 2 c.c. of serum in a centrifuge tube are added 4 c.c. of alcohol and the mixture is centrifuged. To 3 c.c. of the supernatant solution in a graduated centrifuge tube is added 0.5 c.c. of the reagent and alcohol to the 10 c.c. mark. The development of color is read in two minutes in the photelometer. The photelometer reading is then read on the accompanying curve and the milligrams of bilirubin per 100 c.c. serum are obtained from this curve. It is noted that the final volume of pigment solution is 10 c.c. necessitating the use of a smaller cup than is ordinarily used with this type of photelometer. Care must be taken that the pigment solution covers the entire light field.

In Table I is represented a series of forty selected cases in which the values for bilirubin were determined by the Thannhauser modification of the van den Bergh reaction as compared with the new technic. It is clearly demonstrated that the newer technic almost invariably produces a somewhat higher value than the older method. This is to be expected since no precipitates which tend to cause loss of color attributable to absorption are present.

The relationship between the quantitative direct reaction and the quantitative indirect reaction on the same serum is interesting and offers opportunity for further investigation. It is not the purpose here, however, to enter into a discussion as to the reasons for this difference. It is rather the purpose to present an additional use of the photelometer to produce an improved quantitative estimation of bilirubin by following the van den Bergh interpre-

TABLE I

SUMMARY OF CASES IN WHICH THE CONCENTRATION OF SERUM BILIRUBIN WAS DETERMINED BY TWO METHODS

CASE	TYPE OF JAUNDICE	CAUSE OF JAUNDICE	DURATION OF SYMPTOMS	OPERATION	SERUM BILIRUBIN; THANNHAUSER-VAN DEN BERGH METHOD		SERUM BILIRUBIN; NEW PHOTELOMETER METHOD		PATHOLOGY AND FINAL DIAGNOSIS
					INDIRECT	DIRECT	DIRECT	INDIRECT	
1	Obstructive	Cancer; hepatic metastasis	5.5 months	None		17.8	22.7	13.5	Malignancy; obstructive jaundice
2	Obstructive	Cancer of pancreas	1.5 months	Cholecystogastrostomy		20.0	22.0	16.4	Cancer of pancreas; diabetes mellitus
3	Obstructive	Cancer of pancreas with metastasis	7 months	Exploration; dilatation		15.8 19.4	20.4 24.0	14.2 15.0	Cancer of pancreas; obstructive jaundice; extensive metastasis
4	Obstructive	Stones in gallbladder and ducts	Recurrent, 1.5 years	Cholecystostomy and choledocholithotomy for stones; splenectomy		2.8	4.1	3.38	Obstructive cholecystenterostomy
5	Obstructive	Stone in gallbladder and ducts	4 months	Cholecystostomy for stones; choledochostomy for stones		7.7	9.0	6.7	Obstructive jaundice; hepatitis
6	Obstructive	Stone in gallbladder and cirrhosis	3 months	Cholecystostomy for stone		2.5	3.4	2.0	
7	Obstructive	Cholecystitis and stones	7 months	Cholecystectomy for stones		5.0	5.8	4.6	Cholecystitis; cholelithiasis
8	Obstructive	Cholelithiasis; 1 stone	2 weeks	Cholecystectomy for stone		2.5	4.1	2.7	Acute cholecystitis; cholelithiasis
9	Obstructive	Cholelithiasis; stone in common duct	Recurrent, 2 weeks	Choledochostomy for stone; cholecystostomy for stone		8.8 13.6	9.1 12.0	4.9 9.3	Hepatic insufficiency; obstructive jaundice; stone in common duct
10	Mild obstructive	Stone in cystic duct	3 months	Stone removed from duct; cholecystectomy	1.2		1.2	0.5	Acute cholecystitis; cholelithiasis; empyema of gallbladder; stone in cystic duct
11	Mild obstructive	Stone in hepatic duct	3 weeks	Choledocholithotomy; choledochostomy		2.9	4.5	2.0	Atrophic gallbladder; stone in hepatic duct

TABLE I—CONT'D

12	Mild obstructive	Choledocholithiasis	1 month	Choledocholithotomy; drainage of hepatic abscess	1.2		2.0	1.7	Liver abscess; choledocholithiasis
13	Mild obstructive	Secondary anemia; cancer of pancreas	1 year, 2 months of jaundice	Not recorded	1.0		1.5	1.5	Cancer of pancreas; probable carcinomatosis
14	Obstructive	Complete stricture common duct; cirrhosis of liver; ducts obliterated	Recurrent, 9 years	Exploration; inoperable condition		8.32	9.3	5.6	Cirrhosis of liver; complete obstruction common duct
15	Obstructive	Stricture common duct	3 months	Reconstruction of common duct		2.9	3.7	1.5	Stricture of common duct; mild hepatitis
16	Obstructive	Stricture common duct		Hepaticoduodenostomy over catheter		8.6	9.6	5.2	Fistula of common duct into liver
17	Intrahepatic	Catarrh	2 months	None		5.0	5.6	3.0	Intrahepatic jaundice
18	Intrahepatic		3 months	None		12.4 10.5	14.4 10.3	7.4 5.8	Intrahepatic jaundice
19	Hemolytic	Hemolytic icterus	5 years	Splenectomy	3.8			3.8	Hemolytic icterus
20	Hemolytic	Hemolytic anemia	5 years	None		2.0	2.8	2.0	Hemolytic anemia
21	None when test made	Not known; liver normal		None	1.2		1.7	1.5	Irritable gastrointestinal tract
22	None when test made	No organic trouble		None	1.1		1.8	1.6	Chronic exhaustion
23	Sallow	Blood picture indicated pernicious anemia	5 years	None	1.8		1.8	0.7	Pernicious anemia; disseminated sclerosis
24	None	Pernicious anemia	4 years	None	1.5		2.7	1.7	Pernicious anemia; mitral stenosis
25	None	Anemia, severe secondary			1.0		1.5	1.5	Faulty storage of iron; severe secondary anemia
26	None	Severe hypochromic anemia	8 years	Anal dilatation; hemorhoidectomy	1.4		1.7	1.5	Severe hypochromic anemia; hemorrhoids
27	None visible	Anemia	Prolonged	None	2.0		2.2	2.0	Senile macrocytic anemia; lead in urine

TABLE I—CONT'D

CASE	TYPE OF JAUNDICE	CAUSE OF JAUNDICE	DURATION OF SYMPTOMS	OPERATION	SERUM BILIRUBIN; THANNHAUSER-VAN DEN BERGH METHOD		SERUM BILIRUBIN; NEW PHOTELOMETER METHOD		PATHOLOGY AND FINAL DIAGNOSIS
					INDIRECT	DIRECT	DIRECT	INDIRECT	
28	From anemia	Anemia	5 years	None	1.1		1.0	0.5	Subleukemic splenic reticuloendotheliosis
29	Not recorded	Hepatic cirrhosis, Grade 1	2 years	None	3.7		3.6	3.5	Nervous indigestion; mild hepatic cirrhosis
30	None	Secondary anemia	5 months	Exploration; inoperable condition		1.4	2.1	1.5	Epithelioma, Grade 4, of gallbladder; nodules in liver
31	None	Secondary anemia	14 months	None		1.6	1.6	0.8	Carcinomatosis; hepatic insufficiency; anemia
32	Slight	Secondary anemia	1 year	Partial gastrectomy	1.0		1.7	1.3	Ulcerative adenocarcinoma; cancer of pyloric end of stomach with nodal involvement
33	None			Exploration; gastric resection	1.0		1.3	1.3	Carcinomatous gastric ulcer
34	None		6 months	Tonsillectomy	1.1		1.7	1.5	Diabetes mellitus; infected tonsils
35	None	Cholecystectomy, 1930	7 years			1.1	2.2	1.7	Benign hypertension
36	None				1.0		2.1	1.5	Migraine; nervous instability
37	None				1.1		2.0	1.8	Hemorrhoids; dental sepsis
38	None				1.0		1.5	1.0	Chronic nervous exhaustion; migraine
39	None when test made	Past history of splenectomy; cirrhosis of liver; common duct deformed	Prolonged		1.4		2.1	1.5	Sacroiliac arthritis; menopause; hypochromic anemia
40	None when test made	Anemia	3 years	None		2.0	2.0	1.0	Palsy of vocal cord; splenic anemia; phlebitis

tation and terminology of direct and indirect reacting serum bilirubin. An interesting speculation involved in this ratio is whether or not, when high values of bilirubin are encountered and the indirect reaction is preponderant over the direct reaction, this might signify extensive intrahepatic damage rather than high concentration of bilirubin attributable to phenomena of obstruction. This fact might offer clinical evidence as to the prognosis of the disease.

It is felt that this technic offers a more quantitative determination of bilirubin present in blood serum and also offers a knowledge of the ratio of direct and indirect reactions which are produced by the same serum. Without entering into the controversy as to the chemical differences existing between the substances responsible for the direct and indirect reactions, it is a fact that this difference is of clinical significance and is of importance in the determination of the type of disease which is present. The use of this newer van den Bergh reaction is not limited to comparison in the type of photelometer referred to but obviously can be employed in conjunction with other methods of comparison or standardization of color.

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POLYCHROMED METHYLENE BLUE AS A CONSTITUENT OF ROMANOWSKY STAINS*

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1. INTRODUCTION

ALTHOUGH Romanowsky-stained blood smears are so valuable in clinical medicine that they have become routine, unsatisfactory results are commonly obtained. Empirical refinements are numerous, but the principles of the staining technic are poorly understood. There is a similar lack of knowledge with regard to the use of Romanowsky stains for hemopoietic tissues. The growing importance of hematologic examinations as manifested by the increasing numbers of sternal bone marrow punctures and postmortem examinations of bone marrow being performed make it desirable to replace uncontrollable technics by more logical principles of procedure.

There are two general types of Romanowsky stains: (1) those made from polychromed methylene blue and eosin and (2) those prepared from purified, mainly synthetic, dyes. At the present time, of those stains manufactured with polychromed methylene blue as a constituent, Wright's (1902, 1910 a) blood stain is utilized for blood and organ smears, while Wright's (1910 b, 1924) tissue stain serves for the study of sections. Difficulties in the use of these stains are well known. However, apparently because of the facility with which fresh solutions are prepared and the ready availability of other stains on the market, an analysis of unsatisfactory performances has not been undertaken. So little progress has been made in the study of the Romanowsky technic that ordinarily, if buffering of the water does not remedy defects in a stain, little can be done except to prepare fresh staining solutions. The application of polychromed methylene blue solutions to fresh tissue diagnosis (MacCarty, 1923) widens even more the scope of the problem.

Although efforts are continually being made to improve technical results, contributions to the mechanism of action of these stains are almost totally lacking in the literature. A large series of personal experiments on Romanowsky stains has resulted in elucidation of at least some of the questions previously unanswered. In the present paper are described experiments performed with those Romanowsky stains prepared from polychromed methylene blue. A brief review of the history of this subject is a necessary preface to an understanding of the issues involved.

2. HISTORICAL

The men whose stains are in general use today were interested primarily in the microscopic recognition of malarial parasites rather than in the morphology of the blood cells that were seen in the stained smears. Initially, the methods of staining at their com-

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Received for publication, July 28, 1936.

mand were imperfect. Among them, the use of solutions of methylene blue combined with eosin predominated (Chenzinsky, 1888; Sacharof, 1895; Becker, 1901; Engel, 1901; Gautier, 1898; Japha, 1901; Willebrand, 1901). But this stain often failed to demonstrate the malarial parasites, because it did not stain their nuclei, and it did not differentiate clearly the nuclei of the leucocytes from their cytoplasm.

Hematologists were therefore keenly interested when Romanowsky (1890, 1891) announced his discovery that an excellent purplish red stain for the hitherto unstained portion of malarial parasites (now known as the nucleus) could be made by mixing old, mold-covered solutions of methylene blue with eosin. Freshly dissolved methylene blue lacked this action. Romanowsky's stain also differentiated the nuclei and cell granules better than previous stains did, giving the nuclei a purplish red tint and demonstrating the various cytoplasmic constituents in different colors. While the term "Romanowsky stain" is commonly used today, the colors present in a Romanowsky-stained slide are often confused with those produced by eosin-methylene blue stains. With the latter, nuclei stain blue instead of purplish red, platelet granules are poorly demonstrated, and nuclei of malarial parasites are undifferentiated from their cytoplasm. Colored plates in textbooks usually suffer from this error in at least a few cells.

About the time of Romanowsky's discovery, Unna (1891) stained mast cell granules with an alkaline solution of methylene blue which proved later to have a composition similar to that used by Romanowsky. Because of its metachromatic effects, this solution was called polychromed methylene blue. Unna did not intend his stain for use in hematologic work. However, he was the first to recognize that some new substance which yielded a metachromatic effect was formed by "degrading" methylene blue, although Ruge (1900) stated, p. 44: "Twenty years previously R. Koch had already demonstrated that the addition of alkali substantially increased the staining properties of methylene blue solutions."

While Romanowsky's name has been applied to the type of stain being discussed, it was Malachowski (1891) who first learned how to obtain such stains consistently. Independently of Romanowsky and apparently simultaneously, he observed typical metachromatic effects after the use of a single solution of eosin-methylene blue. Since he was unsuccessful in repeating these results with freshly prepared solutions, he investigated various sorts of methylene blue to unite with eosin, and (p. 602) ". . . soon came to the conclusion that the blue stain must be an alkaline one. Finally I found the most suitable color in a very dilute aqueous solution of borax methylene blue. . . . The plasmodia become blue, the granules in them as well as the leucocyte nuclei, red violet. . . . (p. 603) A good uniform stain is obtained when one prepares a mixture of eosin and borax methylene blue and lets the preparation float on it." Malachowski's stain was demonstrated at a meeting in Brieg on June 15, 1890; and at an international congress in Berlin, where Celli, Marchand, Weigert, and others viewed the actual preparations.

This work, however, did not become known, and investigators who tried to duplicate Romanowsky's work experienced much difficulty because usually only eosin-methylene blue stains resulted. The use of certain brands of methylene blue, apparently quite impure, seemed to be satisfactory to some workers (Sacharof, 1895). Consistently good results were not obtained until 1898, when Rosin rediscovered the effects of substituting polychromed solutions of methylene blue for plain methylene blue in Romanowsky stains. At the same time, Ziemann (1898) called attention to Malachowski's experiments and again advocated borax for polychroming.

Nocht (1898; 1899 a, b) elucidated the problem still further. To obtain consistent Romanowsky effects, he combined Unna's polychromed methylene blue solution with eosin. However, he neutralized the excess alkali in the polychromed methylene blue before mixing it with aqueous eosin. Since Nocht could not identify the active metachromatic dye in the "degraded" (polychromed) methylene blue, he noncommittally called it "Rot aus Methylenblau." Maurer (1900, 1902) and Panse (1901) used stains based directly on Nocht's contributions. Laveran's (1899, 1900) stain also was of a similar type, but was polychromed in a way similar to that used originally by Bernthsen (1885), and later by Borrel.

A simplification of technic followed Rosin's (1899 a, b) study of compound stains, in which he found that the eosinate of methylene blue was soluble in alcohol and then was usable as a tissue stain. Independently, Jenner (1899) furthered this trend considerably. Instead of using two separate aqueous solutions to be mixed freshly each time, he collected the precipitate formed when methylene blue and eosin are mixed and redissolved it in methyl alcohol to obtain a single solution which was both fixative and stain. However, since he did not use a polychromed methylene blue, his stain lacked the metachromatic Romanowsky nuclear effects. This final improvement was almost simultaneously achieved by Reuter (June, 1901 a, b) and by Leishman (September, 1901), and later utilized by Wright (1902) and in principle by Giemsa (1904, 1910). In all these technics the slide is fixed by the undiluted stain, then water is added on the slide for staining.

Since Leishman's time numerous Romanowsky modifications have been proposed, but they all consist essentially of polychroming methylene blue solutions in various ways before mixing or precipitating them with eosin. Among the best known is Wright's (1902, 1910 a) stain. Its principal difference from Leishman's is that while Leishman prepared polychromed methylene blue by heating at 65° C. for twelve hours, with subsequent standing for ten days, Wright heated at 100° C. for one hour and used the resulting solution immediately. Later Wright (1910 a) advocated a slightly weaker stain.

Wright's stain early proved unsatisfactory to several investigators who proposed stains more like those of Nocht. Harris (1903) and Marino (1904; 1905 a, b) reverted to two aqueous solutions of the original Romanowsky type. Hastings (1905) and Wilson (1907) prepared stains dissolved like Wright's in methyl alcohol, but made from methylene blue polychromed differently. More recently, Carageorgiadès (1918), Hollande (1916), and Jonesco-Mihaiesti (1918 a, b) advocated stains which are quite similar in principle to the ones already mentioned. The polychroming processes of McJunkin (1920), Proescher and Krueger (1924), and Marie and Raleigh (1924) differ from previous stains only in minor details. Terry's (1928) method, while excellent, is not markedly unlike several previously published ones in which excess alkali was neutralized to stabilize the solution (Nocht, 1899 a; Michaelis, 1901; Goldhorn, 1901; Hastings, 1905; Wilson, 1907). The very recent stain of Groat (1936) does not belong to the type being discussed, as its title might suggest.

Table I summarizes the ways in which methylene blue has been polychromed.

TABLE I

A SUMMARY OF POLYCHROMING PROCESSES

METHODS	ALKALIES USED	TEMPERATURES USED
1. Bernthsen 1885	Ag_2O	Room temperature
2. Goldhorn 1901		
3. Hastings 1905	Borax	Heat:
4. Jonesco-Mihaiesti 1918		water-bath
5. Laveran 1899, 1900	KOH	boil
6. Leishman 1901		arc lamp
7. Malachowski 1890	K_2CO_3, hydrated	ultraviolet lamp
8. Marie and Raleigh 1924	and anhydr.	
9. Marino 1904, 1905		
10. Maurer 1900, 1902	NaOH	
11. McJunkin 1920		
12. Michaelis 1901	$NaHCO_3$	
13. Nocht 1898, 1899		
14. Proescher and Krueger 1924	Na_2CO_3	ACIDS USED FOR NEUTRALIZATION
15. Reuter 1901		
16. Romanowsky 1890	Na_2O_2	1. HAc
17. Rosin 1898		2. HCl
18. Ruge 1900	NH_4OH	3. H_2SO_4
19. Terry 1920, 1928		
20. Unna 1891	$(NH_4)_2CO_3$	
21. Wilson 1907		
22. Wright 1902, 1910	Li_2CO_3	
23. Ziemann 1898		

It should be noted that in the literature on polychromed methylene blue only a single indirect reference (Terry, 1928) has been found to the fact that some of the alkali salts used may be hydrated or anhydrous. The latter, for a given weight of salt, obviously makes the solution much more alkaline than does the former.

Some of these stains have been utilized not only for smears but also for sections, as for example, Leishman's (1904) stain. However, the Romanowsky stain prepared by means of polychromed methylene blue and most widely used for tissues is Wright's (1910 b, 1924) tissue stain, which is also called Wright's megakaryocyte stain. For future reference in this paper, its formulas will now be given.

WRIGHT'S MEGAKARYOCYTE (TISSUE) STAIN

1. Wright's polychromed methylene blue

Methylene blue	1.0 gm.
$NaHCO_3$	0.5 gm.
Distilled water	100.0 c.c.

2. Wright's stock megakaryocyte stain (3:10)

Solution 1 above	3 c.c.
0.2% yel. eosin in CH_3OH	10 c.c.

A proportion of 1 c.c. of polychromed methylene blue was advocated later (1924). These two formulas will be referred to as the 3:10 and 1:10 combinations of Wright's stock megakaryocyte stain.

3. Wright's diluted megakaryocyte stain

Solution 2 above	1 c.c.
Distilled water	1 c.c.

Mix just before use. Run sections down to water, drain, and flood with this diluted stain for ten to fifteen minutes to bring out azurophilic granules in megakaryocytes and platelets. In addition, there are also displayed the various types of granules in leucocytes, colored as in an ordinary blood smear.

To display all of these granules simultaneously, especially those in megakaryocytes and platelets, Wright (1910 b) stated that the tissues must be fixed in 0.9 per cent saline saturated with $HgCl_2$, and subsequently dehydrated not only in alcohol but also in acetone and thick cedar oil, before being imbedded in paraffin. Because the megakaryocyte is probably the least understood cell in hematology today, and because of the vast amount of work done on platelets, the technic for these two elements will be discussed in another paper, where an improved method for their demonstration will be explained.

Since the polychroming of methylene blue is an oxidation resulting in demethylation of the methylene blue molecule (tetramethyl thionin), it is to be expected that different methods of polychroming will result in different end products with various degrees of methylation. Thus such Romanowsky stains contain unknown concentrations of unknown basic dyes. It follows that with these stains it is injudicious to attribute changes in basophilia or properties of the granules to actual changes in the cells themselves, rather than to corresponding differences in the dye contents of the stains. Additional variations are inevitable for another reason also. In practice a given stain for smears is not used even two times successively in exactly the same way. The volumes of stain and water that are mixed, and the length of time the undiluted and diluted stains are allowed to act, are customarily matters of judgment rather than of careful measurement. Therefore, definite standards of color are lacking, and control of intensity of staining is often not feasible.

The experiments to be described were designed to elucidate the action of Romanowsky stains containing polychromed methylene blue on sections and on smears, with the ultimate aim of learning how to control them.

3. MATERIAL

Two types of material were collected as test objects for the stains prepared in these experiments. One consisted of numerous peripheral blood smears from normal individuals, as well as from patients suffering with malaria, leucemia, and pernicious anemia. The second type included a large series of human and animal hemopoietic tissues fixed in various ways. Included were embryonic livers and spleens, and adult bone marrows. They were washed in water, dehydrated in alcohol, and imbedded in paraffin. Details of preparation will be given in a subsequent article on a technic for megakaryocytes and platelets in sections.

Blood smears were made in the usual manner on slides, dried in air, and then fixed by methyl alcohol for three to four minutes. Fixation up to one hour did not appear harmful. Bone marrow smears or touch preparations were fixed up to one hour with Downey's fluid or methyl alcohol. After Downey-fixation, they were washed in running water for one hour and then treated with iodine in 70 per cent alcohol. Fresh alcohol removed the iodine and the slides were run down to water and stained. The alcohol-fixed slides were stained directly after fixation.

The phosphate buffer salts utilized were the primary sodium phosphate and secondary potassium phosphate, mixed in solutions to yield pH's ranging from 5.3 to 8.0. All chemicals were pure (C. P.). Freshly distilled water was available at all times. The various procedures were performed with the accuracy typical of chemical studies.

The initial group of experiments was started on tissues. Pieces of early embryonic pig livers, which show numerous megakaryocytes on an easily differentiated background of hepatic cells and stages in erythropoiesis (Kingsley, 1935 a), were fixed and dehydrated according to Wright's (1910 b) directions. However, Downey's fluid (Wright's saline-$HgCl_2$ plus 10 per cent formaldehyde) proved superior to Wright's as the fixative, and tests for the efficacy of stains in displaying megakaryocyte and other granules in sections were subsequently conducted exclusively on Downey-fixed material, dehydrated and imbedded as described later.

4. FACTORS IN ROMANOWSKY STAINING

When various published methods for staining these tissues proved to be unsatisfactory, an analysis of the technic for both tissues and smears became necessary. Romanowsky stains consist of two parts: dyes and solvents. While a considerable amount of chemical knowledge of the dyes has been accumulated, no investigation has been made of the part played by a given dye in producing a given color effect. It is generally realized that eosinates are formed, but practically nothing has been published directly about their chemical or physical properties. The solvents, except for pH studies, have been neglected entirely, although they have proved to be just as important as the dyes (Kingsley, 1936, 1937). The use of polychromed methylene blue solutions as dyes complicates the problem still more, because it is not known what dyes are present in these solutions or what their concentrations may be. Further-

more, with alkaline solutions, the already unknown dye concentrations are continually changing, due to the progressive oxidizing action of the alkali. So many unknown factors are then present that analysis and standardization are impossible.

When the technic of use of these stains is considered, still other factors enter the problem. If a given dye results in a certain color effect, that same intensity of color cannot be produced by a different concentration of that dye in a stated time interval. In ordinary use, the amounts of stock stain and water that are mixed are not measured accurately, but instead the dilutions are estimated, producing variable concentrations of the dyes. The resulting coloration of identical cells must then necessarily differ from one slide to the next, even when the same stock solutions are used. In addition, the effect of the solvent in the production of the final colors becomes more pronounced at this stage of the technic. Since usually the exact amount of water added is not known, and furthermore, the stock solvent used for fixation has evaporated to an unknown degree before dilution with water, it is impossible to evaluate the rôle of the solvent in such solutions.

For complete control of the usual type of Romanowsky stain, the following factors must be known:

1. Chemical nature of dyes present.
2. Physical properties of dyes present (for solubility data).
3. Concentration of each of the dyes.
4. Color effects produced in the undiluted stain by each dye or its eosinate.
5. Duration of action of undiluted stain.
6. Color effects produced by each dye in the diluted stain.
7. Chemical nature of each constituent of the solvent, including pH of solvent.
8. Rôle of solvent in action of each dye in stain, including effects of volatilization.
9. Duration of action of diluted stain.
10. Selective action of cells on dyes present in stain.
11. Effects of treatment of slides after staining.
    a. Differentiation
    b. Mounting

For blood smears, factor 11 is not concerned except for special purposes. The present paper deals with experiments regarding the first ten of these factors. Later papers will present additional observations.

5. EXPERIMENTS WITH POLYCHROMED METHYLENE BLUE CONTAINING ALKALI

Megakaryocytes were selected as the simplest available means for determining the efficacy of the stains for tissues because when "azurophilic" granules are stained in these cells, the coloration of other hemopoietic cells is also typical for satisfactory Romanowsky stains. Later, blood smears fixed in methyl alcohol proved to be excellent test objects for all types of Romanowsky stains, including tissue stains. In these tests of stains, the tissue factor was controlled by using serial sections from identical blocks of tissue and by taking successive blood smears from a single individual. Wright's (1910 b) directions were followed carefully, so that all other factors were beyond control until some insight had been gained into these problems.

Wright's (1910 b) stock megakaryocyte stain was satisfactory for the first few days after preparation. Then it began to precipitate on the slides in one to three minutes after being diluted with water. This time interval was too short to allow staining to be completed and in addition the precipitate obscured the cells. Both of Wright's stock formulas, the 3:10 and the 1:10, gave equally poor results upon dilution with water.

An occasional satisfactory preparation and the stability of the stock solutions indicated a primary defect in the solvent factor of the diluted stain. For this reason, instead of diluting the stock solution with an equal volume of water, a smaller quantity was tried. After various proportions had failed, it was observed that the following formula made a fair, although weak, stain:

Modified Diluted Megakaryocyte Stain

Stock solution (3:10)	3 c.c.
Distilled water	4 c.c.
CH_3OH	5 c.c.

This formula will be referred to later. Precipitation was rarely seen in this solution, and good results were obtained for several weeks. It is very simple to use, since flooding of the slides and washing with water are the only procedures necessary. This formula is satisfactory for both blood smears and tissue sections. It is unique in being a single solution of a Romanowsky stain, ready to use, yet stable long enough to permit experimentation. Utilization of this principle has permitted analysis of some of the factors in Romanowsky staining (Kingsley, 1936, 1937).

While the solvent factor was adequately standardized by the method above, there was still room for improvement in the time and dye factors. The stain required about ten minutes for action on blood smears, yet resulted in colors much less intense than was desirable, and it was also deficient in metachromasia. On the other hand, Giemsa's blood stain, when used in a formula similar to the modified diluted megakaryocyte stain given just previously, yielded consistently excellent preparations. In analyzing the dye factor, it was therefore believed that the difficulty in obtaining a good stain must be due to the polychromed methylene blue, which varies greatly in its composition, while eosin is a fairly stable chemical compound.

Production of a more suitable polychromed methylene blue was then attempted by substituting various other solutions for Wright's polychromed methylene blue in the 1:10 and 3:10 stock megakaryocyte stains and then using these stocks in the modified diluted stain above. First, two specimens of Unna's solution made by different technicians were tried. The dye factor was totally unsatisfactory.

Second, a fresh sample of Unna's solution was prepared by the following formula:

Unna's Polychromed Methylene Blue

Methylene blue	1.0 gm.
K_2CO_3, anhydr.	1.0 gm.
Distilled water	100.0 c.c.

This was divided into two parts: (1) After standing for twenty-four hours, part one was filtered and substituted in the modified stock and diluted mega-

karyocyte stains. It was satisfactory for forty-eight hours. (2) Since the dye factor was excellent in (1), an attempt was made to hasten the formation and thus increase the concentration of that substance which might be responsible for the metachromatic effect. To accomplish this purpose, the second part of the solution was put into an incubator at a temperature of 60° C. for eighteen hours. The polychromed solution, now twenty-four hours old, was filtered and tested. It was unsatisfactory, as if the useful end-point had been passed. After forty-eight hours, the unheated portion of Unna's solution was likewise of no value. Although tested weekly for three months, no metachromasia was noted.

Third, three specimens of methylene blue polychromed with weak alkalies one year previously and known to have yielded satisfactory results when first prepared, were useless for metachromatic effects when tried in combination with eosin after the year's lapse of time.

Fourth, experiments were then conducted to determine whether the duration of polychroming would change the dye factor sufficiently to result in a Wright's megakaryocyte stain that would not precipitate and yet yield satisfactory colors. Wright's polychromed methylene blue was prepared and placed in a steam sterilizer at a temperature of 100° C. Samples of the resulting polychromed solution were removed from the sterilizer at ten-minute intervals for a limit of ninety minutes. It was observed that, after being heated from forty to ninety minutes, these blue stains yielded apparently stable 3:10 stock solutions with eosin. When these stocks were then diluted, efficient modified diluted megakaryocyte stains resulted. However, when used according to Wright's directions with an equal volume of water, the stains precipitated before good effects could be noted. No marked differences were seen as a consequence of polychroming for various periods between the limits of forty to ninety minutes. The modified diluted megakaryocyte stains remained effective at least for four weeks. When they were tried again after forty-nine days, they failed to show satisfactory results, even when diluted freshly from the 3:10 stocks, which must therefore also have changed. The brand of methylene blue powder used seemed to exert no effect on the unsatisfactory outcome of the final results. These powders were used: Grübler's B.X. (Mayer), Coleman and Bell's medically pure and N.N.S.

From the experiments, it would seem that alkalies acting on methylene blue produce dyes with desirable metachromatic properties, but these substances change still further when exposed to the continued action of the alkali. Heat markedly accelerated this process. That Wright's polychromed blue stain was active much longer than Unna's is probably due to the fact that in the former only 0.5 per cent alkali salt is used, which is just half the concentration present in the latter.

Even the best modified Wright's type of megakaryocyte stain that could be prepared was found to be less efficient in dye content than a diluted Giemsa solution. With the polychromed type of stain, the nuclei were always a muddy purplish blue, and a deep stain of the nuclei could never be obtained. On the other hand, the Giemsa stain, when used similarly, yielded excellent

differentiation of the nuclear structures, although the granules were not as bright as with another stain resulting from these experiments (Kingsley, 1935 c).

6. EXPERIMENTS WITH NEUTRALIZED POLYCHROMED METHYLENE BLUE

Experiments to standardize the dye factor in the polychromed methylene blue solutions were now undertaken. On the basis of the fact that desirable dyes were produced by alkalies during polychroming, it was thought that it might be possible to stop the action of the alkali at the proper time by neutralizing it with acid. Four different solutions of this type proved the hypothesis to be workable: two solutions of Terry's (1928) polychromed methylene blue prepared twelve months previously, and two solutions of Coleman and Bell Unna's solution, from different shipments three years apart. All these were excellent when used with eosin in the modified diluted megakaryocyte stain already described above. The best results were obtained with the Unna's solutions.

These were so much better than those prepared in Section 5 that inquiry was made from the manufacturer about preparation of this better stain. It was learned (Bell, 1934) that for several years Coleman and Bell have neutralized the alkali with hydrochloric acid and added methylene azure A, although the solution is still labeled with Unna's name.

Since these stains were found to have excellent metachromatic qualities even after the lapse of considerable time, it would appear that there was available a basic solution from which to build a satisfactory stain. However, while all four of these solutions were excellent, the results obtained in each instance were different to a slight extent; and their exact dye composition was unknown, even to the manufacturer.

As for the second constituent of the stain, eosin, it was observed that all available eosin powders gave the same excellent results when mixed with a good polychromed methylene blue. The powders tried were: Grübler eosin, w.s.; Coleman and Bell eosin Y and eosin w.s.; and National Aniline eosin yel., certified.

The next group of experiments was designed to standardize the relationship of dye and solvent factors. Various proportions of stain and diluting solution were tried. Less stain (for example 1 or 2 c.c. of stock solution instead of 3 c.c. in the formula for the modified diluted megakaryocyte stain) required too long a period of action or had no metachromatic effect, although such stains precipitated rarely. Increasing the stain concentration accelerated the precipitation. In view of the possibility that the cause of the precipitation might be affected by the pH factor of the solvent, a graded series of 12 phosphate buffers was tried, ranging in pH from 5.3 to 8.0.

These buffer solutions were used to replace the water, both in the original Wright's and in the modified diluted megakaryocyte stains. It was then noted that the lower pH's favored the eosin effect, while predominantly blue colors appeared when the pH went above 7.4. Precipitation was somewhat retarded when a pH between 5.3 and 6.0 was used. However, buffers did not

increase the metachromasia or prevent precipitation. A remarkably high or low pH is necessary to prevent a good stain from exerting its action satisfactorily. Experiments dealing with this point will be published later.

It is interesting to note the following pH's, determined by the quinhydrone electrode:

	pH's
Distilled water, one week old	6.4
Distilled water, 2 days old	6.5
Polychromed methylene blue, Wright's	ca. 9.4
Polychromed methylene blue, Coleman and Bell's	7.4
Polychromed methylene blue, Unna's	over 10.0
Stock megakaryocyte stain, Wright's	over 10.0
Modified diluted megakaryocyte stain (*, Table II)	6.8

In initial experiments it had been observed that the amount of water present was an extremely important factor in precipitation. Insufficient water, however, increases the staining time and causes loss of the metachromatic effect. The proportions of 5 c.c. of CH_3OH to 4 c.c. of water were therefore retained in the diluting solution, and the concentrations of the eosin and polychromed methylene blue were varied in this diluent. Table II shows the amounts used.

TABLE II

COMPLETE ROMANOWSKY STAINS IN ONE SOLUTION MADE WITH POLYCHROMED METHYLENE BLUE AND EOSIN

Figures Are Volume Per Cent

POLYCHROMED METHYLENE BLUE[1]	0.2 PER CENT EOSIN IN CH_3OH	DILUENT[2]
4	14	82
4	20	76
4	21	75
5	20	75
6	20	74
6	30	64
8	30	62
8	32	60
9	40	51
10	40	50
11	40	49
12	45	43
15	45	40
17	45	38
17	40	43
16*	40	44

[1]This is Coleman and Bell's Unna solution.
[2]This diluent consists of methyl alcohol, 5, to water, 4.

The last stain in Table II was excellent for methyl alcohol-fixed blood smears, for megakaryocyte granules in Downey-fixed tissues, and also for Zenker-formol material on which it acted like eosin-azure II. On CH_3OH-fixed blood smears, it consistently yielded excellent Romanowsky stains.

7. DISCUSSION

The advantages of this stain are its cheapness, simplicity, and durability (for at least two months). Furthermore, this single solution is effective in procedures for which three different stains are usually required: a blood stain, Wright's megakaryocyte stain, and Maximow's eosin-azure II. In

megakaryocytes, "azurophilic" granules are not displayed except after fixation in Wright's or Downey's fluids, or in methyl alcohol. However, these granules are not stained in Zenker-formol material by eosin-azure II either. This latter technic is commonly used, so that megakaryocyte and platelet granules have not been seen routinely.

In both sections and smears, the last stain in Table II equals accepted technics in the clear demonstration of azurophilic and other granules. Notable also is the ease of differentiation of the various typical cells. Malarial parasites are brought out, but not as well as by Wright's stain. Nevertheless, the same type of stain, prepared with purified dye crystals (Kingsley, 1935 c), produced effects that constantly equal perfect Wright-stained preparations of malarial blood. Because of the clarity and freedom from precipitation, details which usually are obscured appear prominently, so that the stain will often display granules in cells of acute myeloid leucemias that otherwise require the peroxidase stain for demonstration.

Perhaps most important of all is the fact that this type of formula enables preparation of a stain which is stable long enough to permit determination of the rôle of its various constituents in the final staining effect. The results of further experiments on this problem will be reported later (for brief introduction, see Kingsley, 1936, 1937). It should be noted now, however, that this type of stain obviates the necessity for studying Factors 4 and 5 in the list given in Section 4.

In probing more deeply into the mechanism of action of these stains, a discussion of their chemistry is necessary, but would lead too far afield at the present time. It has, however, been shown that methylene blue cannot be polychromed to yield identical chemical substances consistently, without resorting to methods that are impractical. The conclusions of authorities in this subject are of great practical significance. If polychroming is done by alkalies without heat, less oxidation occurs than when heat is used (Scott and French, 1924). Holmes and French (1926) found many different substances formed under certain conditions. They identified some of these but stated, p. 19: "Still other products of unknown constitution may result, however. . . . The results obtained are too greatly affected by conditions which are not easily controlled, and the products appear to be peculiarly susceptible to further oxidative alteration of an unfavorable nature encountered in drying them, particularly when large quantities of material are being handled."

Years ago, Wilson (1907) polychromed methylene blue with sodium carbonate and then compared the ether and chloroform extracts (these contain dyes requisite for Romanowsky effects) made from the solution at ten-minute intervals during the polychroming. The results showed, pp. 661, 662: ". . . that the amount of material in the ether and chloroform extracts depends on the duration of the boiling and the strength of the alkali; and further that the substances soluble in each solvent do not increase in the same proportion."

To these studies may be added the present experiments, to explain the common experience that aqueous polychromed solutions, initially satisfactory, become useless with increasing age; and that some solutions, originally worth-

less, become progressively better for a period, only to become valueless still later. On the basis of the chemical reactions occurring and the experiments to be reported later on the dyes necessary to yield satisfactory Romanowsky staining, an explanation may be suggested. When directions for the preparation of Unna's and Wright's polychromed solutions are followed, stains satisfactory for a short time result. But the continued action of the alkali present tends to destroy the methylene blue and the more highly methylated oxidation products still present. Yet a definite amount of certain of these must be contained in a stain which is satisfactory in performance when mixed with eosin, so that the solution is a poor stain when the concentrations become subminimal for these dyes. On the other hand, when initial results are unsatisfactory because of insufficient polychroming, age increases the concentrations of these dyes to make better stains.

A study of the rate of oxidation by alkali in the polychroming process was made by Holmes and Snyder (1929), who investigated the effect of pH on the oxidation of aqueous solutions of methylene blue (tetramethyl thionin). Oxidation begins at a pH of 9.5. Below a pH of 11 the rate is slow, and the total oxidation is relatively limited. At a pH of 12, oxidation is rapid and practically complete within a few days. Just as soon as oxidation causes the loss of one methyl group and trimethyl thionin (azure B) forms, the process is accelerated because this dye is decidedly less stable to atmospheric oxidation than is methylene blue. Thus, even at room temperature, oxidation continues in Unna's (pH over 10) and in Wright's (pH 9.4) polychromed solutions. Alkaline polychromed solutions are poor for another reason: one of the eosinates necessary for Romanowsky staining (eosinate of methylene violet) does not form in alkaline solutions (MacNeal, 1906; Wilson, 1907). The same objections are not valid for polychromed methylene blue in which the alkali is neutralized. Terry's (1928) and Coleman and Bell's solutions, which are of this type, proved to be stable for several years in respect to apparent dye concentrations. The influence of progressive alkaline changes is marked only in those Romanowsky stains which are in aqueous solution, as in Wright's tissue stain. When the precipitated eosinates are separated off and dissolved in CH_3OH, as in Wright's blood stain, alkalies which might possibly be carried over on the powdered stain by adsorption can act only after the stain is diluted with water on the slide. Their effect then will be only one of changing the pH and seems of little moment in comparison with the sometimes marked acidity of aged CH_3OH solutions.

Nevertheless, a serious objection can be raised to all polychromed solutions, even to ones that stain satisfactorily, namely, that their dye concentrations are never known. Therefore, standardized solutions are impractical, and unsatisfactory results cannot be analyzed because of the simultaneous presence of too many unknowns. In addition, other experiments to be reported later demonstrate that the amounts of basic dyes requisite for good stains are limited not only in absolute concentrations but also in relative proportions. Yet neither of these qualifications can be met by the use of polychromed solutions. Finally, it is illogical to use unknown solutions when more scientific ones are available (MacNeal, 1925; Kingsley, 1935 c).

The changing nature of alkaline polychromed solutions is probably related also to the controversial opinions regarding the ripening of blood stains of the polychromed type. Dutton (1927) has gathered some interesting data on Wright's stain from replies to a questionnaire concerning the usage of many large laboratories in ripening this stain. Answers showed that some technicians make a practice of ripening the stain, while others find that aging results in a poorer stain. Just what chemical process can occur in the dyes with aging in a purely alcoholic solvent is not apparent. Other than the formation of acid from the alcohol, no reactions have been described until after the stock stain is diluted with water.

Unfortunately, this failure to understand the significance of changes in polychromed methylene blue has influenced certain concepts of morphologic hematology. For instance, it has been regarded as a difficult procedure to stain megakaryocyte granules clearly. The very multiplicity of the methods advocated suggests the difficulties experienced. Petri (1925), for example, insisted on the use of Wright's stain which was between four and fourteen days old to stain megakaryocyte granules, while Ogata (1912) and Petri could not use Wright's stain after Wright's fixative. Basophilia is another concept in which failure to realize technical imperfections has led to controversy. It is well known that merely increasing the pH of the stain tends to increase the basophilia of all the cells. Less apparent has been the fact that variation in the concentrations of the basic dyes present in the polychromed methylene blue may by itself cause different degrees of basophilia from one sample of stain to another; or from one time to another if changes due to alkali can proceed. Thus, different stains may demonstrate different degrees of basophilia in the very same cells.

Various investigators using stains made with polychromed methylene blue must have obtained stains satisfactory for themselves, but of unknown chemical composition, so that confirmation by other workers was impossible. It will be shown later that certain dyes are required for satisfactory Romanowsky staining. It is entirely possible that slight, uncontrollable departures from published directions for preparation caused subminimal concentrations of these essential dyes to be present, with resulting lack of staining by some cellular constituents.

Those stains for blood smears made from polychromed methylene blue have been associated in the United States with the name of Wright so that: "In America of recent years the term Wright's stain has come to be almost synonymous with blood stain, to such an extent that stain companies say it is hard to sell one unless it is so labelled." (Conn, 1930, p. 129.) Leishman's stain, however, is of the same type. Being polychromed at a lower temperature, it should differ in dye content from Wright's, with Wright's containing higher concentrations of the more highly demethylated substances. To some extent these differences are made apparent in the staining effects.

The difficulty in obtaining uniform stains of this type lies obviously in the variability of the polychroming process, and not in the principles involved in the manufacture of the compound dyes. It is not surprising then, to learn

from the Chairman of the Commission on Standardization of Biological Stains that Wright's blood stain has been so inconstant in composition (and presumably therefore in action) that, "As a matter of fact, the manufacturers usually do make it by a different procedure; but sell it labelled Wright stain in order to meet the demand." (Conn, 1930, p. 130.) In addition to the better-known stains, many less-known ones, which are essentially the same as regards their variability in composition and action, were devised to overcome difficulties inherent in the previous stains. While there can be little doubt that these stains gave satisfactory results to their authors, there is likewise little question that their results cannot be duplicated consistently. These stains include those of Bruckner (1908), Carageorgiadès (1918), Harris (1903), Hastings (1905), Hollande (1916), Jonesco-Mihaiesti (1918 a, b), Laveran (1899, 1900), Marino (1904, 1905 a, b), Maurer (1900, 1902), McJunkin (1920), Panse (1901), Ruge (1900), Tribondeau (1916, 1918), Wilson (1907), and Wright (1902, 1910 a, b). A parallel type of stain, made, however, by polychroming toluidin blue instead of methylene blue, was advanced by Epstein (1922). It would appear to offer the same drawbacks as do any of these empirical stains derived from methylene blue by polychroming.

8. CONCLUSIONS

1. Romanowsky stains can be prepared by mixing solutions of polychromed methylene blue and eosin.

2. The composition and therefore the action of the resulting stain depend on the conditions of the polychroming process.

3. Polychroming is a form of oxidation, which as ordinarily performed, decreases the degree of methylation of the methylene blue molecule (tetramethyl thionin). This process occurs in the presence of alkali, is accelerated by heat, and continues progressively until the alkali is exhausted or neutralized. Aqueous alkaline, polychromed solutions therefore change from day to day.

4. Romanowsky stains made from methylene blue solutions polychromed in various ways differ in dye content. Because the polychroming process is practically impossible to control, a given stain of this type, even when prepared according to the published directions, has been found to be inconstant in action and presumably in composition from one sample to the next.

5. The most useful stains are prepared from *neutralized* polychromed methylene blue. These are apparently stable for several years. From them there have been evolved for the first time single solutions which are ready to stain, yet stable long enough to permit experimentation. However, their dye composition and concentration are unknown, and exact duplication seems impossible.

6. Variations in fundamental morphologic characteristics may be produced either by corresponding changes in a given stain in which alkali continues to act after preparation or by using other stains prepared from methylene blue solutions polychromed differently.

7. More unknown factors are encountered during the staining process, when the duration of action of the stock stain and the degree of dilution with water are estimated instead of measured.

8. For these reasons Romanowsky stains manufactured from polychromed methylene blue are not controllable.

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A METHOD OF OBTAINING BIOPSY SPECIMENS OF STERNAL BONE MARROW BY THE USE OF A SPINAL NEEDLE*

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IN GENERAL, sternal bone marrow is obtained for examination, at present, by either of two methods. In the first one, which perhaps is the most widely used, from 2 to 5 c.c. of material are withdrawn by aspiration from the sternum. For the most part this material is blood, but it also contains a surprisingly large proportion of sternal bone marrow cells.

The second method, originally applied to the tibia, is removal of a portion of bone by trephine. Custer has enumerated the advantages of this method as compared with aspiration when used in obtaining sternal marrow cells.

The method here described is believed to offer advantages over both of the above methods. A No. 18 gauge spinal needle is used and the bits of sternal marrow remaining in its lumen upon withdrawal of the needle are spread in a film upon glass slides. Thus, to a degree it is a modification of the trephine method.

DESCRIPTION OF TECHNIC

The patient lies upon the examining table with his head resting comfortably upon a pillow. An area 15 cm. in diameter around the angle of Louis is cleaned (after shaving if necessary) with tincture of iodine, the excess amount of iodine being removed with an alcohol sponge. Having previously scrubbed his hands well and wearing sterile gloves, the physician next anesthetizes the skin at the angle of Louis with 4 per cent novocaine. After a few seconds (five to fifteen) the hypodermic needle is inserted down to the periosteum and care is taken to infiltrate the periosteum well with about 1 to 3 c.c. of solution. After two to three minutes following this a spinal needle (3 inches long, No. 18 gauge) is inserted, with stylet in place, into the manubrium of the sternum, at the angle of Louis. The needle thus is pointed toward the patient's head and is kept at an angle of about 30° from the horizontal of that portion of the sternum.

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Received for publication, August 24, 1936.

When the marrow cavity is entered, there is a certain amount of "give" to the needle, but if the fingers of both right and left hands are braced well against one another and against the chest well, the needle moves only a millimeter or so when the marrow cavity is entered. Once the tip of the needle is inside the marrow cavity the stylet is withdrawn and the needle inserted further for a distance of 0.5 to 1 cm. With the stylet still removed from the needle, the latter then is withdrawn slowly, sufficient bracing of the fingers of each hand permitting the needle to move very slowly and evenly. If there is bleeding following the withdrawal of the spinal needle, a dry sponge may be placed over the skin opening, until the contents of the needle have been examined in the gross.

Once the needle has been withdrawn, the stylet is inserted so that examination of the specimen may be made. In most cases the stylet is inserted for three-fourths of its length before the material within the lumen of the needle is pushed forward. The most satisfactory specimens are those made up practically entirely of yellowish, yellowish pinkish, or reddish gelatinous-appearing flecks. The color will vary depending upon the amount of fat contained in the bone marrow, but the bits of marrow tissue for the most part will be characterized by the gelatinous appearance.

Having this kind of material at the opening of the needle, bone marrow films are made next. Holding the bevel of the needle parallel to the surface of the glass slide, the needle is rubbed back and forth with about 2, 3, or more millimeter strokes until an area about three-fourths of an inch square is covered. The films thus obtained may be allowed to dry in air or may be fixed in methyl alcohol if desired.

DISCUSSION OF THE METHOD

The advantages of the method here described include obtaining of bits of myeloid tissue with the cells in their normal relationship; if desired these relationships could be preserved even better than they are in films, by fixing, embedding, and sectioning. In addition many cells are spread out so evenly that minute cytologic details are clearly evident. Also the procedure is a simple one, quite in contrast to the moderately complex trephining operation. Little healing remains to take place, while whereas trephining soon uses up a considerable portion of the sternum after several biopsies, the present method may be repeated not only frequently but over a long period of time.

The disadvantages of the method seem few: a small amount of tissue, filling of the needle with blood occasionally and the possibility of not reaching a lesion with patchy distribution. All of these difficulties may be overcome however, by repeatedly inserting the needle at different locations during the first or during the subsequent biopsies.

The photomicrographs are of films of bone marrow obtained in the above manner. Fig. 1 shows a portion of marrow with cells in situ, from a normal individual. Note the space occupied by fat globules which were washed from the section in the process of staining. Fig. 2, from a patient with moderately advanced carcinoma of the stomach, shows fewer cells, a moderate amount of serous material and an increase in size of the fat globules. As a matter of

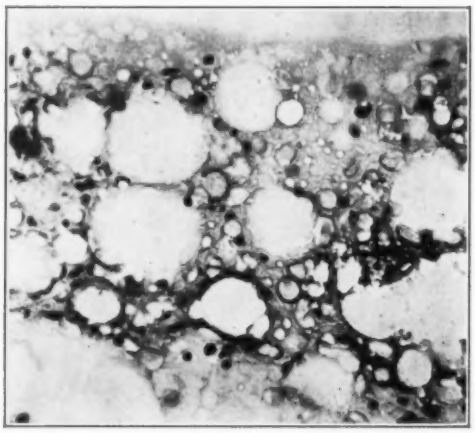

Fig. 1.—Film prepared from normal sternal bone marrow.

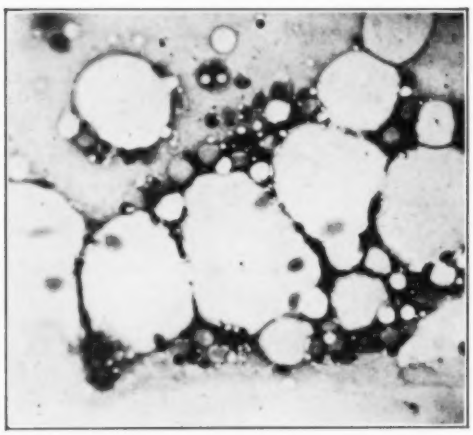

Fig. 2.—Film prepared from sternal bone marrow of patient with anemia secondary to carcinoma of stomach.

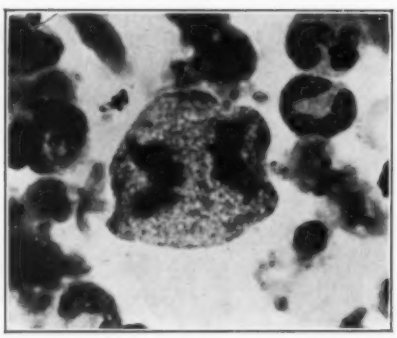

Fig. 3.—Cell in mitosis in sternal marrow film.

fact such a high proportion of fat makes films of this kind of bone marrow difficult to prepare. Fig. 3 is that of a cell undergoing mitosis, the specimen having been obtained and prepared by the present method of procedure. If the sternal bone marrow is hyperplastic (i.e., contains no fat globules), there will be little if any architecture evident in the bone marrow film.

When used in this fashion, the spinal needle apparently scrapes off the marrow from the spicules of bone as it moves forward. Observation of bone in cross-section shows this kind of action while the small bits of bone marrow when spread on the glass slide appear to have been obtained in this manner.

If desired the spinal needle may be rotated upon insertion and withdrawal but there is greater likelihood of the hub becoming unsoldered from the shaft as this joint is not strong in the average needle.

SUMMARY

A method for obtaining biopsy specimens from the sternal bone marrow by use of a spinal needle is described. The advantages of this method are its ease of performance, the obtaining of marrow free from admixture with excess blood, and the fact that the procedure may be repeated frequently as well as over a long period of time.

REFERENCE

Custer, R. P.: Studies on Structure and Function of Bone Marrow; Bone Marrow in Agranulocytosis, Am. J. M. Sc. **189**: 507, 1935.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

WHOOPING COUGH, Correlation Between Sedimentation Rate and Cell Count in Diagnosis of, Gold, A. E., and Bell, H. O. Am. J. Dis. Child. 52: 25, 1936.

Atypical whooping cough is rarely recognized with any certainty in the absence of the pathognomonic whoop. The early clinical recognition of a suspicious condition during the period of its greatest infectivity becomes very difficult and uncertain without the proper support of comprehensive laboratory study.

Many infants and children who have a suspicious cough for an unusual length of time, considered a "cold" of undetermined etiology, may have atypical whooping cough and should be considered to have such a condition until it is proved otherwise, for disorders of this kind can be a dangerous potential factor in the dissemination of the yearly epidemic.

Suspicious persistent cough of a spasmodic and paroxysmal character in cases of catarrhal infection of the upper respiratory tract can closely simulate an atypical attack of whooping cough, even with a typical blood picture of the leucocytic type. This makes a responsible differential diagnosis on the basis of such presumptive evidence difficult and uncertain. In the presence of the influenza bacillus in the ciliated mucosa of the respiratory tract typical signs and symptoms of pertussis are known to be simulated.

A new diagnostic triad characteristic of pertussis of the afebrile and uncomplicated form may be of aid in confirming the diagnosis of suspicious conditions under observation, in the absence of positive findings on cough plates; the triad consists of the suspicious cough, a blood picture of lymphocytosis in association with leucocytosis, and a retarded (normal or subnormal) sedimentation rate. Absence of positive findings does not invalidate a diagnosis of pertussis.

The use of the triad of symptoms present in pertussis has been checked in cases in which there was a typical whoop and by means of positive findings on cough plates in cases of the suspicious cough present in atypical whooping cough, and the sign has been found, on the basis of the evidence presented in this series, to be satisfactorily consistent for employment in improving the reliability of the diagnosis of pertussis.

Such a valuable scientific guide becomes a more reliable method for making an accurate diagnosis of mild or atypical pertussis than the clinical observations based on clinical experience, and the conservative policy of procrastination, the so-called "watchful-waiting" from a tentative diagnosis in the catarrhal stage to a more definite diagnosis in the paroxysmal stage, should be regarded as a responsible factor in the dissemination of the yearly epidemic.

BLOOD, Red Cell Values for Normal Men and Women, Andresen, M. I., and Mugrage, E. R. Arch. Int. Med. 58: 136, 1936.

Previous reports of values for the hemoglobin content, the volume of packed cells and the number of red blood cells of the venous blood of normal adults have been made almost entirely from regions not more than 1,000 feet (304 meters) above sea level.

Information regarding the venous blood of forty normal white men and of an equal number of normal white women living at an altitude of about 5,000 feet (1,520 meters) is given in the present study. Determinations of the number of red blood cells, the volume of packed cells and the amount of hemoglobin, figured from the oxygen capacity, are reported for 240 samples. Corpuscular constants obtained from these values are discussed. The close agreement between the average values for hemoglobin obtained by three methods is shown.

The mean value for hemoglobin of 16.54 gm. per 100 c.c. of blood for men and 14.45 gm. for women and also the mean packed cell volume of 48.35 c.c. per 100 c.c. of blood for men and 43.22 c.c. for women are somewhat higher than values generally reported in other parts of the country. The mean red blood cell counts of 5,420,000 per c.mm. for men and 4,630,000 for women are about the same as the general average compiled by Wintrobe.

The value for mean corpuscular hemoglobin of 30.5 micromicrograms for men and 31.2 micromicrograms for women as well as the mean corpuscular volume of 89.2 cubic microns for men and 93.3 cubic microns for women is higher than Wintrobe's general average.

Figures for mean corpuscular hemoglobin concentration of 34.2 per cent for men and 33.4 per cent for women further confirm previous observations of its constant value in normal human blood.

POLIOMYELITIS, Factors of Resistance in, Hudson, N. P., Lennette, E. H., and Gordon, F. B. J. A. M. A. **106:** 2037, 1936.

Elements concerned in the resistance of the experimental animal Macaca mulatta to acute anterior poliomyelitis were investigated.

A certain degree of resistance was demonstrated in the nasopharyngeal mucosa, although ample evidence was found that pointed to the upper respiratory tract as the portal of entry of the virus. The intestinal mucosa was an effective barrier to infection by virus administered in isolated intestinal loops.

The rôle of the spleen as an organ involved in resistance was studied by the effect of its removal before and after the intravenous injection of virus. Splenectomy seemed to reduce the resistance in two of eleven monkeys and then only when the operation was done before virus injections. In other experiments, virus was found in the spleen in the first twenty-four hours after intravenous or intrasplenic injection. Its disposal in the spleen seemed to depend in part on the contained blood antibodies, since the virus was recovered from spleens of monkeys dying of poliomyelitis only when the organs were perfused. The site of antibody formation was not defined.

Neutralizing antibodies were formed in monkeys "vaccinated" with certain preparations, but their presence was not an indication of effective protection of the animals to intranasal virus.

Natural or artificially induced menstruation and physiologic maturation of monkeys did not lead to the formation of a demonstrable virucidal property of the blood.

Sectioning of the olfactory tracts prevented infection not only after intranasal virus but also after injections of virus intravenously. The selectivity of the virus for this pathway to the nervous system was further indicated by the recovery of virus from the nasopharynx of other monkeys infected by the blood stream.

An explanation was found for the relative resistance imposed as a barrier to the virus between the vascular and central nervous systems. Sublethal doses were made fatally infective by damage to the cerebral cortex by starch injections, and to certain large peripheral nerve trunks by section.

These experiments may be interpreted as meaning that the poliomyelitic infection is primarily and largely of the central nervous system.

A theory of immunity in poliomyelitis logically develops from an analysis of pathogenesis and factors of resistance. We conceive of the virus of this disease as entering the body by the olfactory tract, migrating intracellularly through the central nervous system to the loci of predilection in the cord, and sensitizing the nervous tissue in some way so that it is resistant to re-exposure to the virus. The virus apparently escapes from the nervous tissue and irregularly invades the body, exciting the defense mechanisms of the body with the stimulation of antibody formation. The neutralizing antibody in the natural condition is thus an indication of specific sensitization by extraneural stimulation after nerve cell migration of the virus. Antibodies induced by the artificial conditions so far devised are not necessarily a measure of nerve tissue resistance.

Artificial immunization of man, either active or passive, should take into account the distinctness of the central nervous system in the pathogenesis of poliomyelitis and the signifi-

cance of certain factors of resistance. The interpretation of demonstrable antibodies should be made in the light of these observations, and measures of immunization may well be directed toward the neural features of the disease.

BREAST MILK, Method of Preserving. Scheuer, L. A., and Duncan, J. E. Am. J. Dis. Child. **51**: 249, 1936.

A constant supply of sterile expressed breast milk is of great value for the emergency requirements of a feeding service in a pediatric hospital.

A method for the preservation of breast milk and its clinical use is described.

Over a period of two years, bacteriologic examinations of the specially pasteurized breast milk showed that it remained sterile. Chemical analysis at the end of the second year showed similar values for the specially pasteurized milk and for fresh breast milk.

Examination of the stools of the infants fed the specially pasteurized breast milk showed the fecal flora to be the same as that of the infants fed fresh breast milk.

Seven ounces (178 c.c.) of unpasteurized breast milk with one-half ounce of sterile water was placed in each of a number of ordinary 8 ounce (236 c.c.) formula bottles. Sterile corks were then placed in the bottles but were not pushed down tightly, as they would blow out when the milk was heated. Over each of the corks were placed six layers of sterile gauze, a large piece of cotton and finally a piece of paper. This covering was secured by means of a large rubber band. The bottles were then placed in a bottle rack of eight sections. The rack was placed in the top of a double boiler in cold water which reached to the level of the milk in the bottles. The water was maintained at a temperature of 175° F. for thirty minutes. The bottles were then kept for twenty-four hours at room temperature. At the end of this time the process was repeated and again at the end of forty-eight hours. Following this third heating the corks were tightened in the bottles, the coverings of the corks were removed and the corks were sealed with paraffin. Finally the bottles were stored in the refrigerator at a temperature of 38° to 52° F.

LEAD, New Method for Determination of Minute Amounts of, in Urine, Ross, J. R., and Lucas, C. C. J. Biol. Chem. **3**: 285, 1935.

Reagents: Redistilled water, prepared in a Pyrex all-glass still, should be used for all operations except preliminary rinsings.

1. Glacial acetic acid.
2. Citric acid solution, 10 per cent.
3. Perchloric acid, 72 per cent.
4. Hydrochloric acid, 1 N and 6 N.
5. Ammonium hydroxide, concentrated, 1 N and 0.075 N (or 0.5 per cent).
6. Ammonium oxalate solution, saturated; or powdered ammonium oxalate.
7. Calcium chloride solution, 10 per cent strength.
8. Hydrogen peroxide (30 per cent).
9. Purified carbon tetrachloride. (Chloroform may be used as solvent. It has the advantage that the reagent dissolves in it much more rapidly than in carbon tetrachloride, but the latter is less volatile than the chloroform and for colorimetry, is to be preferred.)
10. Purified dithizone solution in carbon tetrachloride, about 3 mg. per 100 c.c. Dissolve about 4 mg. of commercial dithizone (may be obtained from the British Drug Houses [Canada], Ltd. Toronto, Canada) in 25 c.c. of carbon tetrachloride. Extract with three 25 c.c. portions of 0.075 N (0.5 per cent) ammonium hydroxide. This dissolves the dithizone but not the impurities. Reject the carbon tetrachloride layer. Neutralize the aqueous layer to litmus with 1 N hydrochloric acid and extract the purified dithizone with several 15 c.c. portions of carbon tetrachloride. Dilute to 100 c.c. The solution should be kept in a Pyrex flask in the refrigerator and made up freshly every 3 days.
11. Sodium cyanide solution, 20 per cent.
12. Lead standards. 0.160 gm. of crystalline lead nitrate made up to 1 liter with double distilled water. 2 drops of lead-free nitric acid are added to a 100 c.c. aliquot,

which is stored in a Pyrex vessel. This is Standard 1; 1 c.c. contains 0.10 mg. of Pb; 1 c.c. of Standard 2 contains 0.010 mg. of Pb. This is made by diluting 10 c.c. of Standard 1 to 100 c.c.

The solutions to be used are made from the best analytical grade of chemicals available. Regardless of the claims made on the label all reagents should be tested for their lead content by the following procedure: A solution of the reagent under examination is made up as required for the test and a quantity equal to 3 to 5 times the amount used in the analysis is made just alkaline to bromothymol blue with ammonia. After the addition of a few drops of 20 per cent sodium cyanide the mixture is shaken in a separatory funnel with 1 c.c. of the purified dithizone solution. A cherry red color indicates the presence of lead. Reagents containing more than 0.001 to 0.002 mg. of Pb in the quantities used in the method must be discarded and others tried. In this way the lead content of reagents from different chemical houses may be readily determined; those reagents containing the smallest amounts of lead should be used.

Procedure: Adjust 100 c.c. of urine in the special glass-stoppered tube (or in a glass-stoppered graduated cylinder) to approximately pH 4.5 with bromocresol green indicator by the dropwise addition of glacial acetic acid. Add approximately 0.5 gm. of powdered ammonium oxalate (or 10 c.c. of saturated ammonium oxalate solution) and mix. Then add 2 c.c. of 10 per cent calcium chloride solution and again mix. The lead is in this way completely precipitated by entrainment with the calcium oxalate precipitate. After the mixture has stood for twenty minutes (or overnight) with occasional shaking, the open end of the tube is covered with a cigaret paper held in place by a rubber band, and centrifuged. The supernatant liquid is then discarded and the precipitate is washed with about 15 to 20 c.c. of double distilled water containing a few drops of saturated ammonium oxalate solution. The clump of precipitated calcium oxalate and uric acid is broken up with a glass stirring rod to insure thorough washing. It is then recentrifuged. After the wash water is decanted, the tube is inverted and allowed to drain on filter paper. This removes most of the phosphates which otherwise would interfere later in the procedure. Two cubic centimeters of perchloric acid and 2 drops of 30 per cent hydrogen peroxide are added and mixed with the precipitate by agitation. The mixture is digested for at least twenty minutes on a micro-Kjeldahl digestion apparatus with a glass fume tube. While it is still hot, add 3 drops of the strong hydrogen peroxide (delivered cautiously down the side of the tube), and continue digestion for three minutes. When the mixture is cool, 5 c.c. or more of double distilled water are added to dissolve the perchlorates, followed by 3 c.c. of 10 per cent citric acid solution to prevent the precipitation of traces of phosphates which may still be present. Two drops of bromothymol blue are added and the solution is made *just* alkaline by the addition of 1.5 c.c. of concentrated ammonium hydroxide followed by the dropwise addition of this reagent. If it has been made too alkaline (causing a precipitate to form), adjustment is made with citric acid solution. The digested solutions should not be made alkaline, however, until just previous to extraction, as a precipitate tends to form on standing. If this has occurred they are acidified to dissolve the precipitate and carefully made just alkaline.

If much lead is expected to be present, it is advisable to transfer the solution to a 15 c.c. volumetric flask and use only aliquot portions for subsequent procedures. The clear solution (or a 5 c.c. aliquot) is transferred to a 50 c.c. separatory funnel and 4 drops of 20 per cent sodium cyanide solution are added. At this point the solution should be water-clear and the volume about 20 c.c. The mixture is then shaken vigorously with about 1 c.c. of purified dithizone solution. For extracting very small amounts of lead such as are found in normal urines (less than 0.015 mg.) a more dilute dithizone solution is preferable (one-half strength). The cherry red solution of lead dithizone in carbon tetrachloride is drawn off and the extraction is repeated with 1 c.c. or diminishing portions of the dithizone solution until no further red color appears in the carbon tetrachloride. The lead in this way is completely removed from the aqueous solution. After a little experience with the method this extraction can be made to serve as a rough titrimetric estimation of the quantity of lead present. The supernatant solution, which assumes a yellow color owing to the excess dithizone being dissolved in the alkali, is discarded.

The red dithizone solution, after being washed with about 10 c.c. of 0.5 per cent ammonium hydroxide containing 2 or 3 drops of the sodium cyanide solution (to remove any excess reagent), is made up to a definite volume with carbon tetrachloride and read in the Duboscq colorimeter against a known standard prepared in a similar way. This standard should be extracted at the same time from a suitable quantity of the lead standard. Since green colors are more easily read by some observers than red, it is sometimes advisable to change the color of the recovered lead solutions to the green by shaking with dilute (1:3) hydrochloric acid. The depth of the green color will be proportional to the amount of lead, provided the recovered samples have been washed free of excess reagent.

A distilled water blank should always be run at the same time as the test sample, in order to determine the amount of lead contamination from chemicals, glassware, etc. The amount of lead found in this water blank is finally subtracted from the amount determined for the test samples.

The dithizone solutions should be kept in glass-stoppered tubes away from bright light and read within an hour. Fading of the color is minimized by placing the tubes in the refrigerator until they are read. For determinations of less than 0.015 mg. the total volume of the carbon tetrachloride extracts should be kept within 5 c.c. This necessitates reading these with the microcolorimeter attachment, or against a series of standards in a comparator block.

LEUKOCYTIC BLOOD PICTURE, in Active and Inactive Tuberculosis, Morriss, W. H., and Wilson, G. C. Am. J. Tuberc. 33: 65, 1936.

A study in blood counts in tuberculosis is presented, based on a comparison between the counts made while the patients were clinically active and counts made after discharge from the sanatorium.

In the counts made while the patient was clinically active, 86 per cent of the counts were unfavorable, but 13.8 per cent showed a relatively normal picture.

In 200 patients who were able to work one year after discharge, 49, or 24.5 per cent, had an unfavorable blood picture and 151, or 75.5 per cent, had a favorable blood picture.

A direct comparison of the counts made while the patient was active with a count made when the patient was able to work, showed in the 47 unfavorable counts a favorable change in 32, or 70.2 per cent. In this comparison on 61 cases, 77 per cent had unfavorable pictures while under sanatorium treatment, and 72.1 per cent favorable pictures when they were able to work.

When the comparison was based entirely on the change in the L/M index, the agreement with expectation was a little higher. Thus, of the 43 cases with an unfavorable L/M index while active, 38, or 88.3 per cent, became normal.

BICHLORIDE POISONING, Acute, Chemical Studies of, Sollmann, T., and Schreiber, N. E. Arch. Int. Med. 57: 46, 1936.

The distribution of mercury was studied at autopsy in the organs of three patients who died of typical poisoning from mercury bichloride. The results confirm those given in the few reliable reports of cases in the literature. There is a surprising quantitative uniformity in the data for different patients, although the dosage has some effect. The concentration of mercury is uniformly highest in the kidneys (average, 3.8 mg. per 100 gm. of moist organ, using the medians of the reliable analyses given in the literature); the liver follows, with about one-half to two-thirds of the concentration in the kidneys; then the spleen, with one-seventh; the intestines, with one-ninth; the heart, skeletal muscle and lungs, with about one-fifteenth (0.2 to 0.3 mg. per 100 gm.); and finally the brain, with one twenty-seventh. The concentration of mercury in the blood was between 0.015 and 0.12 mg. per 100 c.c., about one-fortieth of that in the kidneys. The concentration in the ascitic fluid was even lower; that in the bile was variable but always higher than that in the blood and lower than that in the liver. The total mercury content of the liver was about three times that of both kidneys.

BLOOD REGENERATION, Chlorophyll and, Patek, A. J., Jr. Arch. Int. Med. **57**: 73, 1936.

Twenty series of observations on fifteen cases of chronic hypochromic anemia were made with the aim of determining what effect the administration of chlorophyll products might produce on regeneration of the blood.

In five instances the administration of chlorophyll and its degradation products alone did not produce changes in the blood.

In three instances the administration of very large doses of crude chlorophyll with very small doses of iron likewise did not produce change in the blood.

In twelve sets of observations on nine cases the administration of chlorophyll products after a period of medication with comparable amounts of iron was followed by an orderly and significant increase in the concentration of hemoglobin and a second reticulocyte response.

This combined effect was produced when materials were given parenterally as well as orally.

The studies suggested that the body can use preformed pyrrol substances for the building of hemoglobin.

NASAL SMEARS, Cytologic Examination of, in Sensitized and Nonsensitized Persons With Nasal Symptoms, Cowie, D. M., and Buenaventura, J., Arch. Int. Med. **57**: 85, 1936.

Sensitized persons with a nasal discharge have a distinct increase in the eosinophil cells in the differential count of the nasal smear.

It seems safe to say that an eosinophil count in a nasal smear of from 20 to 25 per cent indicates that the patient is allergic.

Eosinophilia occurs in the nasal smears of persons with or without allergy for pollen. It seems to be more marked in persons who have an associated epidermal sensitization.

During an infectious cold there is a reversal in the cytologic picture of the nasal smear of an allergic person. The eosinophil count may be reduced far below that ordinarily noted for an allergic person.

Comparative simultaneous differential counts on the blood smear and nasal smear do not show a definite relationship to each other.

Eosinophilia in the blood, sometimes of marked degree, is not uncommon in sensitized persons in whom no parasitic cause for the eosinophilia can be discovered, and this may be of long duration.

The practical side to the making of differential counts on nasal smears is not inconsiderable. So-called common colds, particularly if they are recurrent, may have an allergic basis (eosinophilous colds). This can often be determined by studies of nasal smears, which may indicate whether or not a prolonged sensitization study is warranted.

Boggy, pale nasal mucous membrane is not a reliable criterion for the diagnosis of allergic rhinitis. When present, it is significant, but it is not always present.

POST-TRANSFUSION REACTIONS, The Minor Blood Agglutinins and Their Relation to, Belk, W. P. Am. J. M. Sc. **191**: 827, 1936.

The autohemagglutinin (panhemagglutinin) is shown to occur frequently in the blood of patients needing transfusions, and to account for the majority of intragroup incompatibilities.

Evidence is advanced that a large per cent of post-transfusion reactions in carefully performed transfusions may be attributable to this agglutinin.

A technic of blood matching designed to detect autohemagglutinin is presented.

The method follows:

Venous blood collected in sterile test tubes is allowed to clot at 37° C., or if previously chilled, is warmed to 37° C. After removal from the incubator, serum is promptly centrifuged and pipetted off to prevent any but slight chilling. The cells are collected in 1.5 per cent sodium citrate in normal saline (or other suitable fluid) and washed twice in saline warmed initially to 37° C. These steps assure a maximum of autoagglutinin in the serum and its removal from the cells. On clean glass microslides ringed with a wax pencil the following

mixtures are prepared: patient's serum and patient's cells, patient's serum and donor's cells, donor's serum and donor's cells, donor's serum and patient's cells. The suspensions are light, about 1 per cent, as an excess of cells masks agglutination. The slides are placed in the refrigerator (5 to 10° C.) for thirty minutes, then removed, one at a time, gently agitated and observed at once under the scope. Agglutination is recorded as from 0 to +4. A second observation is made after about ten minutes at room temperature (or 37° C.) to note reversal of agglutination. The following is an example of the application of the technic from my protocols:

Patient's serum plus patient's cells +4
Patient's serum plus donor's cells +4
Donor's serum plus donor's cells 0
Donor's serum plus patient's cells 0

"Agglutination in patient's serum completely disappeared after ten minutes at room temperature; no rouleaux. Diagnosis: strong autoagglutinin in patient's serum. Advise no more than 100 c.c. blood be given at one time."

INTESTINAL FLORA, and Carbohydrate Intolerance, Gunnison, J. B., Althausen, T. L., and Marshall, M. S. Arch. Int. Med. 57: 106, 1936.

Fecal specimens from persons suffering from a clinical syndrome designated as intolerance of carbohydrate were studied bacteriologically by both qualitative and quantitative methods.

The incidence of *Cl. welchii* ranged from a minimum extreme of less than 100 to a maximum extreme of 250,000,000 organisms per gram. "Stormy fermentation" tests in milk were positive with the majority of specimens, without regard to the number of the organisms present. Plate counts for aciduric organisms varied from less than 100 to 800,000,000 per gram. Dilutions of specimens as high as 1:1,000 to 1:1,000,000 resulted in proteolysis when incubated in beef heart broth. Potato mash, a food causing much distress in patients, was violently fermented by all specimens in test tube cultures. Repeated specimens from a given person often indicated a variation in flora under constant conditions nearly as great as that obtained in different persons.

These results were equally true for specimens from normal persons tested as controls.

Correlations of these results with each other indicate that: (1) The "stormy fermentation" test is not a reliable index of the presence of *Cl. welchii* or of any other predominant types of organisms. (2) The presence of aciduric organisms does not necessarily influence the "stormy fermentation" test, nor does their presence afford any clue as to the incidence of *Cl. welchii*. (3) Proteolytic organisms occur in high frequency and in considerable numbers, without regard to the presence of aciduric types or of *Cl. welchii*. (4) Violent fermentation of potato mash occurs in most specimens, without read to evidence of intestinal symptoms.

No evidence was obtained by the fecal culture methods used in these experiments that *Cl. welchii* bears any direct etiologic relationship to the clinical condition under observation. There was distinct evidence that certain tests which have been suggested for laboratory diagnosis, as well as more elaborate special tests, definitely fail to aid in diagnosis and merely cause confusion.

INSULIN-PROTAMINE COMPOUND, Clinical Observations With, Sprague, R. G., Blum, B. B., Osterberg, A. E., Kepler, E. J., and Wilder, R. M. J. A. M. A. 106: 1701, 1936.

The immediate effect of insulin-P (insulin protamine compound) is much less than that of insulin-R (regular insulin). When the former is used alone, and given as a single dose before breakfast, the meals of the first few days provoke glycosuria, but when the dose is properly adjusted the level of the blood sugar on successive mornings decreases progressively and the elevating effect of meals diminishes until, by the end of from four to six days, a normal level of blood sugar may be attained even in cases of severest diabetes.

Supplementing insulin-P with small doses of insulin-R will shorten the period of obtaining control. Insulin-R should not be mixed in the same syringe or injected into the same site

with insulin-P. It has not been necessary to continue the supplementary use of insulin-R after the first few days in the milder cases, but probably in many cases such supplementary use of insulin-R will be desirable, especially in emergencies. Until more experience has been obtained it would appear that insulin-R will be the insulin of choice when quick action is desirable, as in the treatment of acidosis.

Although insulin-P, in many cases, makes possible effective management of diabetes with only one administration of insulin a day, with less insistence on rigid control of the diet, its careless use or disregard of the diet is attended with danger.

CADAVER BLOOD, Transfusion of, Yudin, S. S. J. A. M. A. **106**: 997, 1936.

Transfusion of cadaver blood was demonstrated in animal experiment and proved its therapeutic value in a considerable clinical material.

Cadaver blood obtained from six to eight hours after death remains sterile and preserves its living properties.

The recipient of cadaver blood is afforded ample safeguards by serologic tests of the blood, a bacteriologic check up as to its sterility, and a careful necropsy.

Because of fibrinolysis, blood of individuals dying suddenly remains fluid and can be preserved for more than three weeks.

The therapeutic effect of cadaver blood does not differ from that of blood of living donors.

The technic of obtaining blood from a cadaver is simple and does not require any special apparatus.

Organization of stations for collection of fresh cadaver blood should offer no difficulties in the larger cities, particularly in the large hospitals for emergency cases. The supply could come from traffic accidents as well as from the medical service where deaths from coronary thrombosis and angina pectoris are not rare.

The method of obtaining the blood follows:

The jugular vein is severed and a glass cannula to which a rubber tube is attached is introduced into each end of the vein. The cadaver is then placed in the Trendelenburg position and the blood is allowed to run into a 500 c.c. flask. The neck of the bottle is stoppered with cotton and the bottle is placed in a refrigerator, where it may be kept for one month.

PROTEINURIA, Bence-Jones, Meyler, L. Arch. Int. Med. **57**: 708, 1936.

The Bence-Jones' protein is found in normal bone marrow, and it is probable that it is a part of the lymphocytes and other leucocytes. When bone marrow is broken off (in cases of tumor metastasis) or when the white cells are destroyed (in leucemia and empyema), Bence-Jones' proteinuria does not appear, as a rule, since the quantity of Bence-Jones' protein produced is too small and is, therefore, broken up in the body. In cases of multiple myeloma the white cells may have the power to produce a large quantity of Bence-Jones' protein, so much that it cannot all be destroyed and is excreted by the kidneys. This is especially true in cases of renal insufficiency.

HETEROPHILE ANTIBODY, Determination as a Practical Diagnostic Aid, Lincoln, M. Clifton Med. Bull. **20**: 13, 1936.

In patients presenting clinical pictures resembling horse serum sickness and infectious mononucleosis, when the diagnosis is in question, a definite conclusion can be reached by the simple laboratory procedure of determining the presence of heterophile antibodies through sheep cell agglutinations.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Synopsis of Diseases of the Heart and Arteries*

THIS small, compact volume was compiled to meet the need for a concentrated treatise on the subject of heart disease. The author has condensed the material found in the more exhaustive standard monographs, with the addition of details and controversial points. To this he has added the more noteworthy advances recently reported in the literature. The book is intended as a manual or guide for students or busy practitioners who are unable to devote the time required for extensive study of a subject important to every physician. The subject matter is presented in an orderly fashion, and numerous illustrations and charts assist in clarification of the more abstract phases. Condensation does not detract from the value of this work to those for whom it was intended. Every helpful method of diagnosis is considered, from special points in history-taking to the use and interpretation of the inductotherm in electrocardiogram. A special section is devoted to Electrocardiography. Each pathological condition is dealt with in a few pages with paragraphs devoted to etiology, pathology, physiology, physical and laboratory findings, and treatment. The book may be highly recommended as a handbook, complete as such, on all the phases of heart disease.

The Thyroid†

THIS book is, perhaps, the most comprehensive text upon the surgical thyroid published within recent times, presenting as it does, not only an extensive survey of present knowledge in both the scientific and clinical phases of thyroid diseases, but the considered experience of the author of many years in their surgical treatment.

While the book is not encyclopedic, nor intended so to be, it will appeal to all who are concerned with or interested in the recognition or management of thyroid conditions, no matter how specialized that interest may be.

As Dr. Bainbridge has said in his Foreword, it is difficult to select the outstanding portions of this text.

The internist and public health worker may, perhaps, turn to the survey of goiter morbidity; the physician at large to the chapters on symptomatology and diagnosis; the surgeon to the sections on operative technic and pre- and postoperative care; the radiologist to the discussion of radium and x-rays. And in each instance, the discussion will be found to be clear and interesting and to represent an ample and extensive experience, adequately digested.

An excellent chapter on the historical aspect of thyroid diseases is appended and an equally useful chapter on nomenclature and classification, together with a bibliography covering thirty-two pages.

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*Synopsis of Diseases of the Heart and Arteries. By George R. Herrmann, M.D., Ph.D., Professor of Clinical Medicine, University of Texas. Member Association of American Physicians, American Climatological and Clinical Association, American Society for Clinical Investigation, American Society for Experimental Pathology, and The Society for Experimental Biology and Medicine. Fellow American Association for the Advancement of Science, American College of Physicians and The American Heart Association. Miembro Correspondiente Extrajero de la Sociedad Mexicana de Cardiologia. With 88 text illustrations and 3 color plates, cloth, 344 pages. Price $4.00. The C. V. Mosby Company, St. Louis, 1936.

†The Thyroid: Surgery, Syndromes, Treatment. By E. P. Sloan, M.D. Edited by members of the Sloan Clinic. With a Foreword by Wm. Seaman Bainbridge, M.D. Cloth, 475 pages, 99 illustrations, $10.00, Charles C. Thomas, Springfield, Ill.

The numerous illustrations are excellent in themselves and excellently reproduced.

All in all, this volume may be recommended without reserve as a well-conceived, well-written, and well-considered text constituting a valuable contribution upon a highly important subject.

Recent Advances in Endocrinology*

THAT a third edition of this book should be called for a year and a half since the publication of the second edition is ample evidence of its value and usefulness.

The present edition has been extensively revised and rewritten and includes such recent developments as the protamine compounds of insulin, the newer knowledge of the estrogenic compounds, the recent studies on the thymus and pineal glands, and many other developments in the field of endocrinology.

This third edition may be expected with confidence to maintain the standard and popularity of the others.

A Manual of the Common Contagious Diseases†

THAT this useful book has reached a second edition is not surprising for it presents in a clear and readable manner a comprehensive survey of the common contagious diseases of use to the medical student, interne, and physician alike.

The text has been extensively revised and in some sections rewritten, while much new material has been added.

This little book may be recommended as an authoritative and useful manual which might with profit become the physician's desk companion.

California Medical Association. Cancer Commission Committee Studies‡

THIS publication, as the title implies, is a group of studies, reports, conclusions, and recommendations submitted by the Cancer Commission Committee of the California Medical Association, created in 1931. One of the chief aims of the Cancer Commission, in the presentation of these reports, has been to develop a better agreement in the profession, and a wider diffusion of knowledge of the diagnosis and adequate treatment of early cancer in the various regions of the body. The need for coordination of experience and ideas of therapists in the various special fields, particularly surgery and radiology, is stressed. Various committees have reported on radiology, gynecological tumors, breast tumors, skin and mouth tumors, eye, ear, nose and throat tumors, genitourinary tumors, chest tumors, bone tumors, connective tissue tumors and leucemias, tumors of the central nervous system, gastrointestinal tumors, cancer of the rectum, anus, and rectosigmoid, and thyroid tumors. No more than a few pages are devoted to each of these sections, containing an outline of the symptoms and signs which assist in making an early diagnosis, and the various methods of treatment. This pamphlet will be a valuable addition to the library of diagnostician, specialist, surgeon, and radiologist.

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*Recent Advances in Endocrinology. By A. T. Cameron, Professor of Biochemistry, University of Manitoba. Cloth, ed. 3, 458 pages, 65 figures and 3 plates, $5.00. P. Blakiston's Son & Co., Philadelphia, Pa.

†A Manual of the Common Contagious Diseases. By Philip Moen Stinson, Assistant Professor of Clinical Pediatrics, Cornell University Medical College. Cloth, ed. 2, 437 pages, 53 engravings and 3 plates, $4.00. Lea and Febiger, Philadelphia, Pa.

‡California Medical Association. Cancer Commission Committee Studies. Paper, 123 pages. J. W. Stacey, Inc., San Francisco, California, 1936.

Recent Advances in Allergy*

THOSE familiar with the previous editions of this useful and valuable book will welcome the third edition which has been extensively revised and rewritten to bring it into line with the many advances in this field.

While the book will naturally take its place upon the reference shelves of the allergist, it should prove of special interest and value to the physician desirous of keeping abreast of the many advances in this far-flung field.

Dr. Bray's volume may be recommended as presenting a comprehensive and authoritative summary of the field of allergy in which the work of American as well as foreign investigators has not been neglected.

Cardiac Output and Arterial Hypertension†

AMONG the problems which have long engaged the attention of the physiologist and the clinician as well is that concerned with the development of methods for the determination of the cardiac output.

Among the better known methods is that proposed by Marshall and Grallman in 1928. In this volume the author presents a partial report of his investigations as Fellow for Cardio-Vascular Research in the Mount Sinai Hospital of New York. The work described disputes the validity of the Marshall-Grallman method and describes a method considered more accurate and reliable and discusses also, on the basis of results secured with the author's method, the work of the heart in arterial hypertension and the pathogenesis of nephritic hypertension.

Chemical Procedures for Clinical Laboratories‡

THIS book is intended to serve as a laboratory guide for the students in biochemistry in the New York Postgraduate Medical School. It will without doubt, however, find a much wider field of usefulness and should serve as a valuable and useful guide to laboratory workers in general.

The methods presented are based upon experience and practical trial and are presented in such detail as to be clear to those not particularly well informed in this field.

The book is divided into four main sections devoted to the blood, urine, gastrointestinal secretions, and miscellaneous biological fluids among which are included milk, spinal fluid, and effusions and other body fluids. There is a useful appendix containing reagent formulae and other useful laboratory data. It may be safely predicted that this book will attain a wide circulation and find a wide field of usefulness.

Statistical Methods for Research Workers§

THAT so specialized a book should rapidly reach a sixth edition is prima facie evidence of its practical value to the audience to which it is addressed.

The present edition has been revised and rewritten to incorporate the advances made in this field.

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*Recent Advances in Allergy. By George W. Bray, Late Asthma Research Scholar. The Hospital for Sick Children, London, ed. 3, cloth, 517 pages; 107 figures, 4 colored plates, $5.00. P. Blakiston's Son and Co., Philadelphia, Pa.

†Cardiac Output and Arterial Hypertension. By Sidney A. Gladstone, M.D. Cloth, 56 pages, $1.00. Privately printed.

‡Chemical Procedures for Clinical Laboratories. By Marjorie R. Mattice, Sc.M., Assistant Professor of Clinical Pathology, New York Post Graduate Medical School. Cloth, 520 pages, 88 figures, 2 colored plates, $6.50. Lea and Febiger, Philadelphia.

§Statistical Methods for Research Workers. By R. A. Fisher, Sc.D., F.R.S. Ed. 6. Cloth, 339 pages. Oliver and Boyd, Edinborough.